

CARL MAR: MÖLLER:

MYCORRHIZAE AND NITROGEN ASSIMILATION

with special reference to mountain pine
(*Pinus Mugo* Turra) and Norway spruce
(*Picea Abies* (L.) Karst)

Mycorrhizer og Kvælstofassimilation,
med særligt Henblik paa Bjergfyr og Rødgran
(Dansk Resumé)

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MYCORRHIZAE AND NITROGEN ASSIMILATION

WITH SPECIAL REFERENCE TO MOUNTAIN PINE
(*PINUS MUGO* TURRA) AND NORWAY SPRUCE
(*PICEA ABIES* (L.) KARST)

BY
CARL MAR:MØLLER

INTRODUCTION.

In a supplement number to „Tidsskrift for Skovbrug“ P. E. MÜLLER in 1903 published a paper entitled „Om Bjergfyrrens Forhold til Rødgranen i de jyske Hedekulturer“ (On the relation of European mountain pine (*Pinus Mugo* Turra) to Norway spruce (*Picea Abies* (L.) Karst) in the Jutlandic heath plantations).

The author here sets up the hypothesis that the promoting effect which an admixture of mountain pine has on the growth of Norway spruce in heath plantations is due to a capacity of the mountain pine to assimilate the free nitrogen of the air. The seat of this nitrogen assimilation is assumed by him to be the dichotomous mycorrhizae of the mountain pine, which may often by repeated and close bifurcation form up to pea-sized nodular mycorrhizae, which may to some extent resemble the root nodules of alder. It is only the species of pines which have such dichotomous mycorrhizae, and precisely the pine species exhibit an exceptional ability to grow with dark-green needles and relatively luxuriantly in such a meagre soil that the Norway spruce is unable to thrive there. The pines, like the spruce and most other trees, in addition to simple unbranched, probably also have cluster-shaped or racemose mycorrhizae, but according to P. E. MÜLLER (1903, pp. 14,31) the dichotomous mycorrhizae are entirely predominant on pronounced meagre soil “while the normally branched mycorrhizae are absent or only occur sparsely” (1903, p. 14) (translated from the Danish).

Furthermore MÜLLER (1903, p. 21) thinks he has rendered probable by his anatomical investigations that the cause of the dichotomy is not to be found in the usual fungal mantle, since on quite young long-roots he has observed emerging verruciform dichotomous processes devoid of fungal mantles. He is therefore of opinion that the cause of the dichotomy "must be a parasitic influence of a different kind, and the capacity of these rudimentary roots as ectotrophic mycorrhizae must be a secondary phenomenon" (translated from the Danish).

By the parasitic influence of another kind he either thinks of early appearing endophytic hyphae or of bacteria as in *Alnus* and the *Leguminosae*.

In 1920 the Danish Heath Society at the instance of P. E. MÜLLER invited competitors to write a prize essay on the subject: "To investigate and demonstrate experimentally whether the mycorrhizae of the mountain pine and the closely related Scots pine are capable of absorbing the free nitrogen of the air".

In the same year I took up this task, laboratory facilities being placed at my disposal at the Plant Physiological Laboratory of the Royal Veterinary and Agricultural College by the late Professor FR. WEIS, Ph. D., to whom I also owe thanks for interest and support in other respects. I am likewise indebted to my chief at that time, professor in forestry JOHS. HELMS, for valuable impulses, and to the then assistant at the Plant Physiological Laboratory, the present Professor K. A. BONDORFF, for great interest and advice in the technique of microbiology.

In two tempi, on December 31, 1920, and December 31, 1922, respectively, I submitted a preliminary paper, which was found worthy of receiving two-thirds of the prize.

In the journal issued by the Heath Society (1923, p. 159) a note is found about this preliminary reply, which in the main merely states that I had failed to demonstrate by experiments that the mycorrhizae of the mountain pine were capable of absorbing the free nitrogen of the air.

My results available at that time (not stated in the journal of the Heath Society) I had summarised as follows in my paper:

- 1) In the synthesis experiments carried out by me I did not succeed in producing "true" mycorrhizae. A number of fungi which I had isolated from mycorrhizae had during the experiments proved to be either parasites or saprophytes on the roots, where the parasitic fungi had been able to

form thickenings, in which numerous intracellular hyphae could be observed under the microscope.

- 2) My experiments with the isolation of fungi from the mycorrhizae showed that a number of different fungi contribute to the formation of the external fungal mantle. The species become the less exacting in regard to nitrogen the poorer the sites are. Fungi isolated from mountain pine roots on wind-swept sands have particularly modest requirements.
- 3) The mountain pine itself, i. e. when raised under perfectly sterile conditions, is unable to assimilate the free nitrogen of the air.
- 4) Mycorrhizaless mountain pine plants may sustain life with surprisingly small quantities of nitrogen.
- 5) Ordinary mycorrhiza-bearing specimens of mountain pine taken in nature will thrive in culture experiments with surprisingly small amounts of nitrogen.
- 6) In the experiments it was impossible to demonstrate any unmistakable nitrogen fixation in mycorrhiza-bearing mountain pine plants cultivated in nitrogen-free sand cultures.
- 7) Mountain pine plants from very poor sites have a lower percentage content of nitrogen than mountain pines of the same age from a good site.
- 8) Mountain pine plants from very meagre localities have comparatively few and poorly developed dichotomous mycorrhizae, while plants from more favourable sites have a larger number of and more profusely developed dichotomous mycorrhizae.
- 9) The mycorrhizal fungi do not seem to accompany the seed, since unsterilised seeds of mountain pine made to germinate on a sterile substrate yield plants free from mycorrhizae. Mycorrhiza-producing forms of fungi must accordingly occur nearly everywhere in the soil.
- 10) The roots of the mountain pine, the mycorrhiza-bearing as well as (and especially) the mycorrhizaless, will thrive with a surprisingly small supply of oxygen.

Before submitting the last part of my paper I had become acquainted with ELIAS MELIN's preliminary reports on some successful synthesis experiments with pine and spruce (Melin 1921 a and b) and his more complete report on synthesis experiments with larches (1922), by which it had been shown

that the mycorrhizae of conifers may be produced by the inoculation of sterile plants with 1) (for pine and spruce) various non-definable fungi isolated from living mycorrhizae (probably, however, Hymenomycetes) and 2) fungal tissue isolated from fruit bodies of *Boletus luteus* for Scots pine and *Boletus elegans* for larch.

It appeared from the preliminary report on the synthesis experiments with pine and spruce that the species of fungi might to some extent replace each other as producers of mycorrhizae. Further two of the forms which most commonly produce dichotomous mycorrhizae on pines had also been able to produce simple mycorrhizae in larches.

These data were pointed out in my paper.

Drawing support from the aforementioned points 3—8 as well as from the fact pointed out by MELIN that the various species of fungi may to some extent replace one another as mycorrhiza-producers, I wrote that "the probability of the view hitherto held, viz. that the mountain pine, similarly as the Leguminosae, is able to assimilate the free nitrogen of the air by means of its dichotomous mycorrhizae, and that this is the reason why it is able to promote the growth of the more nitrogen-requiring Norway spruces in places in which they are otherwise unable to grow, must be regarded as invalidated" (translated from the Danish).

As an explanation of the aid given by the mountain pine to the Norway spruce I instead pointed out the fact that it is less exacting than any other woody plant known in this country. "Altogether, as far as I can judge, the modesty of its requirements both of oxygen and nitrogen is the property that more than any others allows the mountain pine to become the pioneer among the species of trees".

I further referred to the possibility that the mycorrhizal fungi of the trees may have a special ability to decompose, consume, and possibly pass on organic nitrogen compounds, which, judging from the ordinary mode of living of the Hymenomycetes, does not seem improbable.

Considering the fact that the mountain pine is able to help the Norway spruce to start growth in raw heath soil, although the same fungi may form the mycorrhizae of the two species, I thought the case sufficiently explained by the circumstance that *the less exacting mountain pine is able to create a forest environment*

beneath it. Hence the effect should simply be similar to that found when nurse trees of birch on grass-bound soil are able to help towards starting the growth of a beech culture.

The effect must be assumed to be due to the following facts:

- 1) The mountain pine kills the heather with its shade.
- 2) It protects the soil against desiccating draught and intense sun-light.
- 3) It aerates and prepares the soil by the constant traffic of its widely spreading roots, which periodically (in dry periods) die away.
- 4) It causes a decomposition of the heather mor (raw humus) through the effects 1—3.
- 5) It throws off a layer of needles the decomposition of which is more favourable than that of the Norway spruce needles.

Although these results and viewpoints must be regarded as being of some importance in practice, various circumstances have prevented me from submitting the finished work till now.

First of all I have been occupied by other work. Further, after the publication of MELIN's papers I had planned and commenced certain new investigations on mountain pines which it would take a long time to complete. The work included thorough investigations of the influence of mountain pine on the soil beneath it.

These investigations have only recently been finished.

In the present paper is presented a complete report of my investigations (with the exception of certain parts no longer of interest). At the same time the results of the more significant recent works dealing with the mycorrhiza question are reported and discussed so as to give a total picture of our present knowledge of the subject.

I am greatly indebted to Mr. F. PIPER, forester to the Danish Heath Society, and Mr. K. KIERKGAARD, State forester, for the interest and care with which they have assisted me by collecting soil samples, forwarding plant material, and the like.

I am also indebted to the Danish Heath Society for letting me have the free use and right to publication of my prize essay.

To the Rask-Ørsted Foundation I am much indebted for granting me the means for the translation into English, and to professor S. O. HEIBERG of Syracuse for kindly revising the translated text.

I. THE ANATOMY OF THE MOUNTAIN PINE AND THE NORWAY SPRUCE MYCORRHIZAE

As regards the general main features of the anatomy of mycorrhizae and the historic development of our knowledge of this, the reader is referred to SARAUW (1893), to the introductory sections of MELIN's papers (1923, 1925), and to HATCH (1937 pp. 9—38).

A. AUTHOR'S OWN ANATOMICAL INVESTIGATIONS

As material I used preparations made by myself as well as sections made by other workers, i. a. a number of hand-cut sections of mountain pine made by Professor Dr. KØLPIN RAVN and a collection of microtome sections of pine and spruce mycorrhizae executed by the Bohemian PEKLO and kindly lent to me for use by P. E. MÜLLER. My own sections were stained according to the safranin-gentianviolet-orange method and fixed with chrome-platinum-acetic acid. KØLPIN RAVN's sections were stained in various ways, i. a. with orseillin B B and aniline blue. The procedure by which PEKLO's sections were stained I no longer recollect.

The roots of the young plants, the formation of the mycorrhizae.

Even in one-year old seed-bed plants of mountain pine and Norway spruce the development of mycorrhizae is in full progress. The tips of the long-roots are covered by the well-known root cap and free from fungi. The cells in the primary cortex are fairly loosely deposited, and even immediately below the root tip the intercellulars often begin to be filled with fungal hyphae, so that the "Hartig net" (see fig. 1), described and explained at length i. a. by SARAUW, will soon form, though as yet there need not be any fungal mantle present at all. Now issuing from cortical cells with a "Hartig net", now from uninfected cells, root hairs are often found near the tips, especially in spring (cf. TUBEUF 1896 and 1902, p. 70). Both in mountain pine and in Norway spruce these are sometimes seen to be penetrated by rather large, mostly hyaline hyphae, which are provided with clamp cells and continue into the root (though here without clamping) and, probably with a destructive effect, penetrate

the lumina of the external cortical cells, though, according to my observations, in a rather sporadic and casual way (cf. SARAUD p. 59) without such coils, anastomoses, or plectenchymatous formations as were observed and figured by A. MØLLER (1903).

With the exception only of the tips of the long-roots and a small minority of roots entirely free from mycorrhizae, the fungal mantle keeps the greater part of the younger roots closely covered, in the pine irrespectively of whether we are concerned with dichotomous or racemose mycorrhizae. The picture is quite similar when viewed under the microscope.

The anatomy of the fully developed mycorrhiza

is clearly demonstrated by fig. 1. Externally the so-called fungal mantle (fig. 1 a) is seen, which forms a very beautiful false parenchyma. A few hyphae are seen to project freely from the external side of this parenchyma; as to these hyphae, which may be more or less vigorously developed, see further below (p. [8]). Immediately under the fungal mantle there occur *a few dark-coloured, mostly flattened, dead epidermal cells (b) or external cortical cells, mostly penetrated by hyphae*, and below these the normal living cortical cells (c), which are usually filled with tannin and often seen to be provided with a well developed nucleus and entirely enclosed in hyphae, the aforementioned *Hartig net*.

According to my observations these hyphae keep quiescent and neither in mountain pine nor in Norway spruce send haustoria into the cells, as previously stated by REES (1885) for Scots pine (and *Elaphomyces*) and by MELIN (1923) for Scots pine. It will also be seen that the hyphae do not extend further down than to the endodermal cells (d), recognisable by their often suberised inward-turned cell walls, which are coloured red by safranin, and their higher content of plasm. Often farther outward, but at any rate *here*, the hyphae cease to advance.

The endodermal cells are probably always intact. And the fortification afforded by their closely fitting, often suberised ring is probably the cause of the standstill.

It remains to be pointed out that the intercellular network is only faintly represented in the root tip of the simple or racemose mycorrhizae, although the external fungal mantle may continue

unaltered all the way round. However, in a certain region, right under the tip and inside some few cell layers, there is a crowding of big, readily stainable nuclei, almost entirely filling the small cells, in which also numerous starch grains are deposited. It is evidently a phenomenon corresponding to the meristem of the vegetative cone in uninfected roots, and this tissue is presumably especially viable and protects itself and its nearest surroundings against the intrusion of the fungus. Finally it may be mentioned that the picture just outlined is, indeed, the typical picture, but there are numerous deviations from it. Thus the external fungal mantle as well as the intercellular network may present a widely different development, not only from one species of tree to the other or from plant to plant, but also from one part of the root to the other in the same plant. Both may be entirely absent, or the external fungal mantle may, though rarely, be absent and yet a well developed network be present, or the fungal mantle may be well developed and the intercellular intruding hyphae may be few and rather superficial, which is a more common feature.

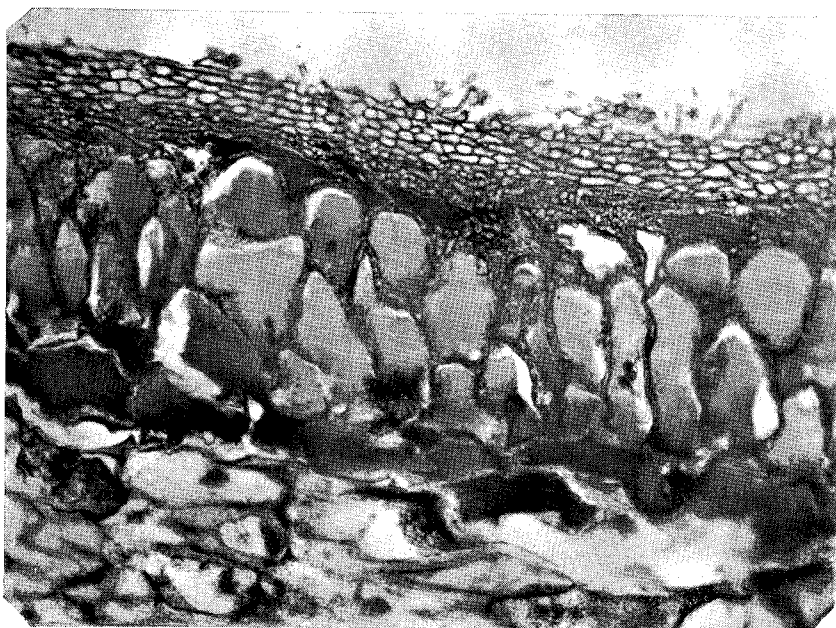
The external hyphae are as a rule of a bright yellowish or slightly brownish colour, more rarely dark-brown or dark-green, while the intercellular hyphae are light-coloured and clear.

As a further illustration I have added fig. 2—4.

As described in the above the anatomy has always been observed by me both in mountain pine and Norway spruce. The microscopic picture of dichotomous and racemose mycorrhizae in pines shows no constant differences which may explain the morphological disagreement. Dichotomous mycorrhizae occur, indeed, though sparsely, in pine plants produced in a sterile way (cf. i. a. MELIN 1925, figs. 23, 24).

It appears from fig. 1 that the hyphae, also the intercellular hyphae, have a thickness of $1-3\ \mu$ — that is to say the same thickness as many free-living hyphae in the surrounding soil. At the top of fig. 1 (in the middle) a hyphal apex is seen projecting from the fungal mantle. It is fairly thin itself, but its basal part is joined to a thicker group of hyphae swollen by anastomosis. That the plectenchyma-forming hyphae are on the whole nearly always somewhat anastomosed, is fairly certain. Is is distinctly observable in places where a piece of the fungal mantle happens to be cut loose, so that it appears freely in the field.

Moreover the Hartig net proper is most frequently formed

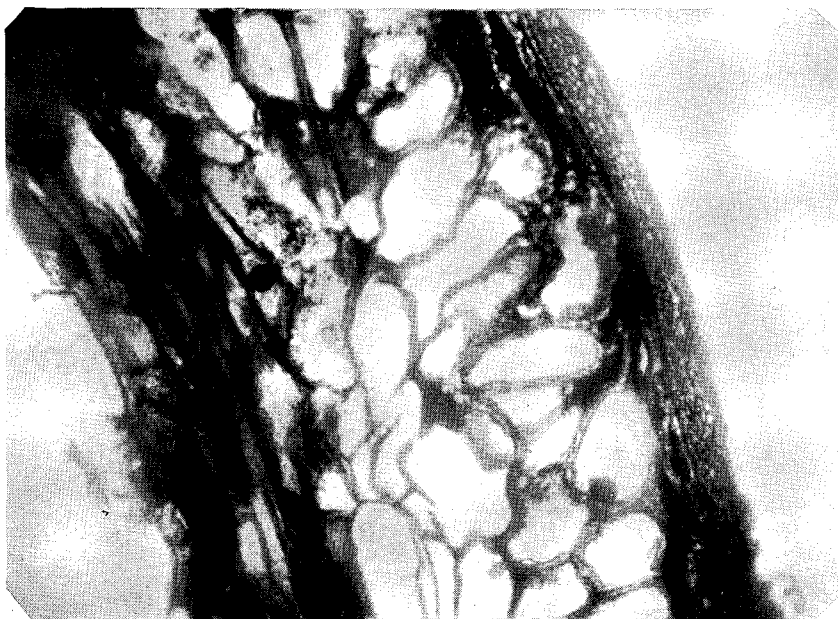


C. M. M. FOT.

Fig. 1. Longitudinal section of a young dichotomous mountain pine mycorrhiza from a two years old nursery plant. (x 400).

a: fungal mantle; b: dead epidermal or external cortical cells;
c: living cortical cells; d: endodermis; e: central cylinder.

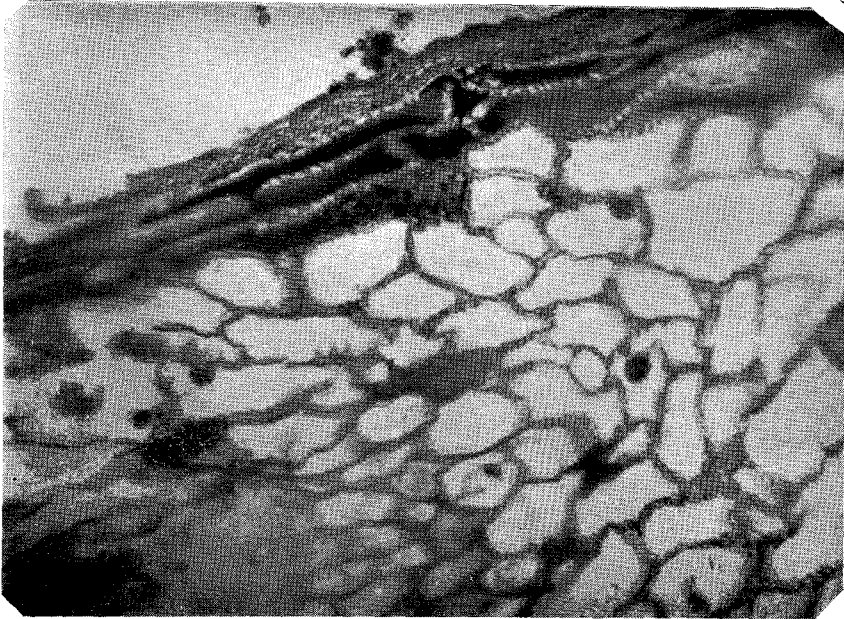
Længdesnit af ung dichotom Bjergfyrmykorrhiza fra 2-årig Planteskoleplante (x 400)
a: Svampeskeden, b: døde Epidermis-Celler eller ydre Barkceller,
c: levende Barkceller, d: Endodermis, e: Centraleylinderen.



C. M. M. FOT.

Fig. 2. Longitudinal section of a mountain pine mycorrhiza. The hyphae appear in several places typically as intercellular strings of pearls. Below, right, a living nucleus in an ensheathed cell. The outermost cortical cells are dead and collapsed. They are coloured blackish-red by saffranin. (x 400).

Længdesnit af Bjergfyrmykorrhiza. Hyferne viser sig flere Steder typisk som intercellulære Perlesnøre. Forneden til højre ses en levende Kerne i en omspunden Celle. De yderste Barkceller er døde og sammensunkne. De farves sortrøde med Saffranin. (x 400).



C. M. M. FOT.

Fig. 3. Oblique section of a Norway spruce mycorrhiza near the tip of the root. Here also strings of pearls, living nuclei and collapsed cortical cells are seen. Small fragments of a Hartig net are seen from the surface, but not quite distinctly. (x 400).

Skraat Snit af en Rødgranmycorrhiza nær Rodspidsen. Her ses ogsaa Perlesnore, levende Kærner og døde Barkceller. Smaa Brudstykker af Hartigsk Net skimtes. (x 400).



E. HELLMERS FOT.

Fig. 4. Single dichotomy in mycorrhiza of mountain pine. Observe the "beard" of hyphae. (x 20).

Enkel Dichotomi paa Mycorrhiza af Bjergfyr. Bemærk det af de frie Hyfer dannede Skæg. (x 20).

by somewhat anastomosed irregular hyphae, which can be easily observed when fragments of the network are loosened in thin sections.

It is obvious that the lacking fructification renders it difficult to estimate on an anatomical basis which species of fungi it is that produce the mycorrhizae.

A great many vigorous mycorrhizae in all species of conifers from all sites — in pines irrespectively of whether they are dichotomous or racemose — are enclosed by a "beard", readily visible to the naked eye, of mostly white hyaline hyphae, most frequently gathered in bundles or network and abundantly provided with clamp-cells, which are considered to be a fairly unmistakable specific character of the Basidiomycetes, of which various species have also been found to be mycorrhiza-producers in forest trees. These hyphae, in a particularly marked degree in dichotomous pine mycorrhizae, may bind the root (the mycorrhiza) to the surrounding soil and its humus particles, so that in light, but still humous, sandy soil, where the phenomenon is especially conspicuous, many up to walnut-sized lumps may attach to a drawn-up root, as observed and described by P. E. MÜLLER (1903) and several other authors.

The intimate way in which the hyphae may bind the sand to the roots is reminiscent of the activity of the root hairs.

So far my own investigations, — whose results agree in the main with the picture now generally accepted, though neither in pine nor in spruce was I able to observe intracellular fungal hyphae apart from those which penetrate and probably in most cases kill the external cortical cells.

B. OBSERVATIONS BY OTHER AUTHORS OF INTRACELLULAR HYPHAE IN CONIFERS

A number of earlier authors (REES 1880, SARAUF 1893, STAHL 1900, A. MÖLLER 1903, TUBEUF 1903, MANGIN 1910, FUCHS 1911, PEKLO 1913, LAING 1923, MELIN 1923) have recorded the finding of intracellular hyphae in the living cells of the cortex in the roots of conifers, and several of these authors have described a decomposition and digestion of these hyphae in the cells.

A comparison of the descriptions show, however, that they exhibit a fairly great mutual disagreement, as some examples will show.

A. MÖLLER (1903) describes intracellular hyphae in the cortical cells: "They pass through the cell wall from cell to cell, branching abundantly and forming anastomoses" (l. c. p. 324), "The hyphae are only found in the cortical cells already turned brown . . . The intercellular Hartig net is not connected with the intracellular mycelium" (translated from the German). In a first paper (1902) MÖLLER says i. a.: "If an entirely sound, normally developed plant . . . is found to be penetrated by fungal mycelium to such a great extent, and if this observation is then found to be confirmed almost without exception in readily growing plants, then the idea obtrudes itself even more urgently than in the case of the ectotrophic mycorrhizae that a special physiological importance might be attached to these mycorrhizae" (translated). Later, however, (1903, p. 323) he is inclined not to place confidence in an endophytic symbiosis, and FUCHS and MANGIN, who have themselves observed similar intracellular hyphae in Scots pine to those observed by MÖLLER, are of the same opinion. MÖLLER says further (1903) that the endophytic hyphae are only found in certain dark-coloured, slightly thickened areas of the youngest mostly one-year old roots, and that the intercellular mycorrhizal hyphae is by far the most predominant.

TUBEUF, however, is positive that he has found actually endotrophic mycorrhizae in Scots pine, and reproduces (drawn) a "transverse section of a young pine in which also the lumina of the cortical cells are filled with fungal hyphae. These are connected with the intercellular fungal network and occur only where the external fungal mantle is reduced" (translated).

MÖLLER's and TUBEUF's descriptions and figures agree very poorly with each other, and the two authors are, indeed, very sceptical about each other's views.

MÖLLER says as follows (1903, 325): "I have never observed features in the pine which correspond to those described by TUBEUF. I am not, of course, contesting the correctness of the observation, but merely propose a reexamination".

And similarly, TUBEUF (l. c. p. 80), mentioning that part of MÖLLER's paper which appeared before his own paper cited here, says that actually nothing can be seen in the accompanying figure (as indeed Möller admits in his paper quoted later, referring to the poor technique of reproduction).

TUBEUF goes on to say: "Since, when the paper was submitted,

I had the opportunity of seeing the glass diagram myself. I can say that in this it was not possible, either, to see whether the hyphae occurred in the lumen or in the membranes, and whether the cells photographed still contained a nucleus and plasma" (translated).

So "doctors disagree".

Personally I agree with A. MÖLLER in his objection that it is difficult to believe in the drawing reproduced by TUBEUF. I, too, have never observed similar pictures — except, perhaps, in cases in which, especially in moderately thick sections, the Hartig net (with fragments of the cell wall) has happened to be loosened so that it may be viewed freely in the lumen, in which case a picture arises that may resemble that given by TUBEUF.

In 1913 the Bohemian PEKLO reported that in both pine and spruce mycorrhizae he had found nearly constantly occurring large quantities of endotrophic hyphae — not only in the cortical cells, but also in the very meristem of the vegetative cone, in the endodermis, and in the root tip (i. e. the apical part of the mycorrhiza with omission of the meristem). He likewise found that a digestion of the hyphae is taking place. They fall to pieces and are dissolved.

In his summary of these cytological observations (p. 266) he points out the constant endophytic infection of the roots in pine and spruce, and goes on: "The author must, at any rate, interpret the statements by FUCHS and other authors, according to which the endotrophic mycorrhiza only occasionally occurs in pine, as being dropped. It is, no doubt, likewise due to imperfect methods of preparation that the same author, like MANGIN, believed he had only found the endotrophic infection in old cell layers of the mycorrhizae . . ." (translated).

To this I must point out that in PEKLO's collection of preparations which in 1921 was handed over to me for examination by P. E. MÜLLER it was absolutely impossible to observe the phenomena mentioned by PEKLO in 1913 although P. E. MÜLLER had informed me as to what PEKLO had seen in them. Nor was Mr. OVE ROSTRUP, m. sc., whom I showed the preparations, able to see the phenomena.

Actually much speaks against the correctness of the observations.

Notably the great number of parasitic or symbiotic fungal hyphae indicated to be occurring in the meristem of the vegetative cone itself is at the outset contrary to nature.

The meristematic cells are, it is true, thin-walled and accordingly easily accessible quite mechanically; but on the other hand they nearly always lie well protected, beneath several layers of uninfected cortical cells and are probably the most vital cells of the root tip, which should, indeed, render them suited to defend themselves against attacks. Nor should we, according to their whole character, expect that they would be capable of carrying out their mission with the appertaining nuclear divisions, etc., if such a great number of endotrophic hyphae had penetrated into the cell cavities as stated by PEKLO.

I must be very sceptical as to PEKLO's statements, which have not been confirmed by other investigators either, and I cannot help imagining the possibility that we are here concerned with a wrong interpretation of the precipitates, fragments of protoplasm, and the like often found in preparations, or that the lines of refraction between different precipitated substances which occur in any preparation, may have been regarded as hyphae, similarly as loosened parts of the network. Perhaps, finally, the fine striae always present, which the cutting may produce in the stained plasma or in precipitated colour substance, etc., may have served to create a wrong impression. This would seem to be indicated by the fact that PEKLO in his description of the endodermis very often speaks of "more or less regular striation by hyphae, which often show a distinct connection with the adjacent cells" (e. g. p. 253, line 14 from below), and on p. 252 (line 13 from below) he says: "In thin and faintly stained sections the cells often appear to be finely transversely or longitudinally striated" (by the hyphae). — (translated)

MELIN (1923) gives a detailed account of the anatomic observations made by him, accompanied by drawings.

In addition to a Type I, corresponding in the main to my observations for dichotomous mycorrhizae in pine (i. e. a chiefly ectotrophic type), he describes an ectendotrophic Type II, in which, besides the usual external fungal mantle, the Hartig net, and a faint intracellular infection of the outermost tannin-containing, partly dead cells, a so-called "digestive layer" is

found, situated just inside the layer, marked b in my fig. 1, of tannin-containing, often dead, epidermal and external cortical cells. This digestive layer (MELIN 1923, p. 95) usually consists of three cell layers, and is characterised by an abundant endophytic infection. The hyphae will soon break up into fragments and their contents will be digested. A Hartig net (reseau) will have developed except in the cell layer nearest to the endodermis. The strongest infection will be active in the two innermost cell layers.

Hyphae are lacking in the endodermis. The process in the digestive layer is described i. a. as follows (l. c. p. 96 et seq.): "Die Zellen sind hier in den erwachsenen Parteien mit grossen hyalinen Körpern von sehr verschiedener Form und Grösse versehen. Bald sind sie ziemlich regelmässig kugel- oder eiförmig, bald unregelmässiger geformt, oft langgestreckt und mehr oder weniger gebogen. Sie liegen gewöhnlich in der ganzen Zelle zerstreut (manchmal so dicht, dass sie pseudoparenchymatische Anhäufungen zu bilden scheinen). Bisweilen sind sie aber an einer Stelle konzentriert Der Inhalt der Körper lässt sich mittels der verwendeten Methoden nicht färben, dagegen treten die Wandungen durch Orseillin-Anilinblau sehr deutlich hervor. Auf den mit Eisenhämatoxylin oder Dreifachfärbung behandelten Schnitten treten sie überhaupt nicht hervor. . . .

In den meisten Zellen sind Pilzhypen nicht zu entdecken (Tafel, Fig. 5), in anderen Zellen hingegen sind solche zu finden (Tafel, Fig. 6) und sie verlaufen in diesen Fällen in den peripheren Teilen der Zelle. Oft sieht man diese Hypen in Verbindung mit den eben beschriebenen Körpern (Taf. I, Fig. 6). Die Hypen sind ebenso wie diese ganz hyalin und der Inhalt lässt sich mit Orseillin-Anilinblau nicht färben. Sie haben ganz dünne Wandungen, sind gewöhnlich 2 μ dick und besitzen keine Septen.

Die Kerne dieser Zellen haben gewöhnlich eine unregelmässige Form, und zwar oft eine mehr oder weniger eckige. . . . Das Protoplasma besteht aus einem dünnen Belage um den Kern und die Zellwand herum und erscheint im Mikroskop nur undeutlich von den Gerbstoffvakuolen begrenzt.

Die hyalinen Körper haben sich aus Pilzhypen gebildet. . . . Die Veränderungen, denen die intrazellularen Hypen unterworfen sind, entsprechen denjenigen, welche in den Verdauungs-

zellen bei Orchideen stattfinden (MAGNUS 1900, BURGEFF 1909), auch wenn der Verlauf ein ganz anderer ist. Durch die enzymatische Tätigkeit der Zellen wird der Zelleninhalt der Hyphen zweifelsohne aufgelöst und verdaut, während gleichzeitig die Hyphen selbst in grössere oder kleinere Stücke fragmentiert werden...“

From several passages in Melin's papers it appears that the ectendotrophic mycorrhizal form thus described is not only found to occur commonly on dichotomous mycorrhizae of Scots pine and mountain pine, but also on the other types of mycorrhizae in both Scots pine and mountain pine, Norway spruce, birch, and asp (e.g. 1923 pp. 106—108).

In the passage quoted above reference is made to a number of figures, all of them drawings. It would have been desirable that the account should have been accompanied by clear microphotographs on this important point, but this is not the case. The microphotographs which accompany the work show nothing in this respect.

MELIN indicates himself that it is due to the fact that, unlike earlier investigators, he used staining with orseillin-aniline that he was able to observe the decomposition of the hyphae in the digestive layer, etc., since he always arrived at poor results if he used other staining methods, e.g. HEIDENHAIN's iron hematoxylin and FLEMING's tricolouring, “da sich in diesen Fällen namentlich die dünnen Haustorienhyphen im Innern der Zellen sich nicht oder nur schwer wahrnehmen lassen“ (1923 p. 89).

While MELIN's synthesis experiments have later been abundantly confirmed by other investigators, this, however, is not the case with his observations of the regular occurrence of endotrophic mycorrhizae in the above-mentioned species of trees.

E. V. LAING (1923) found no endotrophic mycorrhizae on mountain pine, Norway spruce, Sitka spruce, or silver fir, whereas he found them on Scots pine, whence he describes them as follows:

“In these roots there is a well-defined zone of fungus-containing cells distant a cell or so from the endoderm. The mycelium within these cells is coiled, and from this layer of cells fungal filaments proceed in a radial direction towards the exterior. This radially-penetrating mycelium is much finer than the other, which is irregularly swollen and coiled.”

It will be seen that this description does not quite agree with that of MELIN.

In a later paper (1927) MELIN mentions, indeed, intracellular hyphae, but as of less frequent occurrence. About his mycorrhizal form E occurring on soil favourable for the formation of mycorrhizae he says (translated from the Swedish):

"Numerous richly branched hyphae 4—6 μ thick and with an irregular vacillating course occur intracellularly. . . . Whether the intracellular hyphae fall to fragments or not, cannot be decided at present, for these mycorrhizae have only been examined in material cut by hand."

Apart from this, the digestive process is not mentioned in the paper.

KELLEY (1930) mentions well developed »Verdauungszellen« in *Pinus echinata*, *Larix Kaempferi* and *Picea Abies* and gives a drawing (fig. 2,7) which however to me does not seem convincing.

His paper is on certain points surprising. In *Picea Abies* f. i. he found no Hartig net but still a general infection of outer cortex and pericycle. Further he mentions infection of the central cylinder in *Pinus sylvestris*, *Picea Abies* a. o. as a typical feature, which at any rate does not harmonize with the observations of most other authors. Of course I can not contest the correctness of his descriptions but I find it difficult to accept them as typical for the species.

MCARDLE (1932), who i. a. has examined 128 series of sections of white pine and Norway spruce, found ectendotrophic mycorrhizae in a few cases only and observed no fragmentation. ("No trace of the "digestion" could be found" p. 308).

LINDQUIST (1932) has found ectendotrophic mycorrhizae on Norway spruce from vegetationless mor, but his description leaves doubts. His fig. 6 perhaps shows intracellular hyphae, namely 3 specimens seen in transverse section near the right wall of the cell on the extreme left, but nothing can be seen of the strong fragmentation of these hyphae, mentioned by the author p. 16 l. 4 from below. What he regards as hyphae in

fragmentation (fig. 6) can, according to my view, only be a part of the cell plasma appearing in the section, as is evident from the intimate contact between the presumed fragmenting hyphae and the undoubted nuclei in all the cells of the section where anything is found which may be conceived to be hyphae.

At any rate the picture given by LINDQUIST does not resemble the digestion process described by MELIN (1923).

HATCH & HATCH (1933) state, after the pattern of MELIN, that they have synthetically produced typical ectotrophic mycorrhizae on *Pinus strobus* with twelve of 37 tested fungi. A simultaneous occurrence of endotrophic mycorrhizae is not mentioned.

A. B. HATCH (1937) in a comprehensive investigation speaks only of ectotrophic mycorrhizae.

ENDRIGKEIT (1937), who has studied the mycorrhizae of conifers at different seasons of the year and on varying soils, found only ectotrophic mycorrhizae apart from one case, the endotrophic character of which he regards as pathological.

MODESS (1939, 1941), as MELIN's assistant, produced fresh synthetic mycorrhizae by means of a series of Hymenomycetes and Gasteromycetes, but says nothing about the type of the mycorrhizae formed.

BJÖRKMÄN (1942), who has made thorough investigations on the conditions for the development of mycorrhizae in pine and spruce, has, it is true, found ectendotrophic mycorrhizae to a limited extent, but refers to them as follows (l. c. p. 173): "Ektendotrofe Mykorrhizen mit einem dünnen Hyphenmantel aber dicken HARTIGSchen Netzwerk und einer kräftigen intracellularen Infektion (der Pilz ist wahrscheinlich mehr oder weniger parasitisch) wurde vorzugsweise bei ziemlich schwachen Pflanzen in Humusformen mit geringer Stickstoffmobilisierung (in Fichtenwäldern von VACCINIUM-Typ und in geschlossenen Beständen flechtenreicher Kiefernwäldern) angetroffen."

A microphotograph (l. c. fig. 5) of an ectendotrophic mycorrhiza shows a picture which can hardly be interpreted as a

decomposition and digestion of the intruding hyphae. Nor is a digestive process mentioned.

It appears in different ways that the author only attaches importance to the ectotrophic mycorrhiza.

BJÖRKMANS 600 cuts are stained with orseillin-aniline. The other authors quoted as a rule do not indicate staining method or they have used other methods than the orseillin-aniline.

The digestive zone and the digestive process of the ectendotrophic mycorrhiza form a main feature in Melin's description of the presumed mutualistic symbiosis between the root of the tree and the fungus, and have thence passed as a fact into most hand- and text-books published since then.

According to the above statements I think it must be regarded as doubtful whether this feature in the general mycorrhizal picture should be maintained.

II. OCCURRENCE OF DIFFERENT TYPES OF MYCORRHIZAE ON DIFFERENT TYPES OF SOIL

A number of Swedish investigations (HESSELMAN & MELIN 1927, BJÖRKMAN 1940, 1942) have shown that the mycorrhizae of the pine and the spruce develop most profusely on normal mor¹⁾ in the central Swedish mossy coniferous woods and in North Swedish mossy coniferous woods in an active humus state (e. g. of the *Geranium* type).

The mycorrhizae develop poorly, however, in North Swedish mossy coniferous woods of the *Vaccinium* type and the *Dryopteris* type, in lichenous pine woods (i. e. of low quality class), and on typical mull. Mycorrhizae occur sparsely on recently drained peaty soil.

Similarly P. E. MÜLLER and FR. WEIS (1906, p. 277) found for 1 year old beech plants only a very weak development of mycorrhizae on ordinary beech mor, whereas on looser types of beech mor treated with chalk a profuse formation of mycorrhizae was observed simultaneously with a rich nitrification.

As to the mountain pine I have made similar observations: The development of mycorrhizae is best in vigorously growing

¹⁾ Mor is the modern term for raw humus.

mountain pines on moderately good heath soil, and poorest in starved mountain pines on wind-swept sands.

The general development of the mycorrhizae is always well reflected in the number of dichotomies.

The following figures should be viewed in this light.

Of 76 self-sown plants of varying size from Harreskov Sande (very meagre wind-swept sand) only forty had an ascertainable dichotomy, in no case particularly well-defined. The remaining 36 plants were not inferior to the forty in regard to development and weight in relation to age.

Of forty plants collected on another occasion in the same place, eighteen had no dichotomous mycorrhizae.

In a nutrition experiment with plants from Harreskov Sande (cf. p. [57]) all the plants used were numbered and described. As regards the presence of dichotomies on the roots the following classification was used with the following result:

| Plants | Dichotomies | | | |
|-----------------------------|-------------|-----|---------|------|
| | none | few | several | many |
| Large | 2 | 8 | 3 | 2 |
| Medium-sized | 7 | 13 | 3 | 2 |
| Small | 12 | 5 | 1 | 2 |
| Total of sixty plants | 21 | 26 | 7 | 6 |

It should be added that the large plants were often about ten years old. Their needles were green and healthy irrespectively of the dichotomy, which had no influence, either, on the relation between age and weight.

Similarly, of the 65 plants used in the same experiment for determining the nitrogen contents at the start, 17 of the 25 small, 10 of the 25 medium-sized, and 3 of the 15 large plants had no dichotomy at all of the roots.

Some figures from a good nursery in the Vilsbøl dune plantation may be mentioned for comparison.

| Seed-bed plants | Dichotomies | | | | |
|---------------------|-------------|-----|---------|------|-------|
| | none | few | several | many | total |
| One year old | 17 | 27 | 19 | 37 | 100 |
| Two years old | 1 | 15 | 26 | 58 | 100 |

In this connection the following observation is likewise of interest.

In Harreskov Sande it was remarkable that rambling roots very often seek the remains of the many roots which have previously died.

The new thin roots as a rule make their way right down through the older thick decaying roots, which may be close-packed locally with profusely developed mycorrhizae, whose "beards", i. e. the hyphae issuing from them and provided with clamp cells, could be seen to grow luxuriantly out through the cells of the old root: The cell walls proved under the microscope to be thickly set with round holes all over, evidently originating from cellulose-fermenting bacteria.

From this it would be natural to infer that the mountain pine plant by means of its mycorrhizae derives benefit from its own dead remains; however, in the dead roots not penetrated by fresh roots entirely similar clamp cell hyphae are found in large numbers, living freely as saprophytes and showing a very similar picture of the destruction of the root remains.

It is therefore quite conceivable that the hyphae issuing from the mycorrhizae may be saprophytes on one side and epiphytes on the other, while the pine plant draws benefit from the presence of organic colloids and from the organic decompositions affected by the fungi, just as the case would be with a bacterial decomposition taking place outside the root.

It does not necessarily follow that the nutritive elements pass through the hyphae to the root.

III. ISOLATION EXPERIMENTS AND SYNTHESIS EXPERIMENTS.

As stated above, such experiments have been made in a superior way by MELIN (1917—1925), who found mycorrhizal symbiosis between the following species of trees and fungi:

Larch: *Boletus elegans*, *B. luteus*, *B. variegatus*; *Amanita muscaria*; *Tricholoma psammopus*; *Cortinarius camphoratus*.

Mountain pine: *Boletus granulatus*, *B. luteus*, *B. variegatus*; *Lactarius deliciosus*; *Russula fragilis* (i. e. *R. fallax*); *Tricholoma virgatum*; *Cortinarius mucosus*.

Scots pine: *Boletus badius*, *B. granulatus*, *B. luteus*, *B. variegatus*; *Amanita muscaria*; *Lactarius deliciosus*; *Russula fragilis* (i. e. *R. fallax*); *Cortinarius mucosus*.

Norway spruce: (*Boletus luteus*); *Amanita muscaria*; *Lactarius deliciosus*; *Cortinarius balteatus*.

Quaking asp: *Boletus scaber*, *B. rufus*.

Birch: *Boletus edulis*, *B. scaber*, *B. rufus*; *Amanita muscaria*; *Tricholoma flavobrunneum*.

After MELIN, and using his technique, a number of investigators (McArdle 1932, Hatch & Hatch 1933, Doak 1935, Modess 1939, 1941, and others) have made mycorrhizal syntheses, i. a. the following:

Weymouth pine: *Boletus bovinus*, *B. castaneus*, *B. granulatus*, *B. luteus*; *Cantharellus cibarius*; *Amanita muscaria*; *Lactarius deliciosus*.

Mountain pine: *Boletus flavidus*, *B. subtomentosus*; *Amanita mappa*, *A. muscaria* var. *umbrina*, *A. pantherina*, *A. rubescens*; *Lactarius helvus*, *L. rufus*; *Tricholoma albobrunneum*, *T. imbricatum*, *T. pessundatum*; *Rhizopogon roseolus*.

Scots pine: *Boletus flavidus*, *B. bovinus*; *Amanita mappa*, *A. muscaria* var. *umbrina*, *A. pantherina*, *Lactarius helvus*; *Tricholoma albobrunneum*, *T. pessundatum*, *T. vaccinum*; *Clitopilus prunulus*; *Entoloma rhodopolium*; *Rhizopogon luteolus*, *R. roseolus*.

Norway spruce: *Boletus flavidus*; *Amanita mappa*, *A. muscaria* var. *umbrina*, *A. pantherina*; *Lactarius helvus*; *Tricholoma albobrunneum*, *T. imbricatum*, *T. pessundatum*; *Lycoperdon gemmatum*; *Tricholoma personatum*; *Clitocybe rivulosa* var. *angustifolia*, and *C. diatrea*.

It will be seen that the mycorrhiza-producing fungi are all Basidiomycetes, either *Boletus*, *Agarics*, or *Gasteromycetes*.

Also the mycorrhiza-producing fungi isolated by MELIN from mycorrhizae, viz. *Mycelium radialis silvestris* α , β , γ , and *Mycelium radialis abietis*, may to a great extent replace each other as mycorrhiza-producers, as it appears e. g. from the following synoptic scheme (MELIN 1923, p. 200):

| | Pine | Spruce | | Larch | |
|----------------------|--------------------------------------|------------------------|-----------|------------------------|-----------|
| | Mycorrhiza-producer of the 1st order | Mycorrhiza-producer of | | Mycorrhiza-producer of | |
| | | 1st order | 2nd order | 1st order | 2nd order |
| M. R. silv. α | + | — | + | — | + |
| M. R. silv. β | + | + | — | + | — |
| M. R. silv. γ | + | + | — | — | — |
| M. R. abietis | + | + | — | — | — |

In nature mycorrhiza-producers of the 2nd order rarely form mycorrhizae, as they cannot hold their own in the competition with the mycorrhiza-producers of the 1st order. The column marked Pine must be considered to refer (MELIN 1924) to the same extent to mountain pine and Scots pine.

The type *M. R. silvestris* α is for various reasons (growth picture, smell, etc.) regarded by MELIN as identical with the mycorrhiza-producing *Boletus* species of the pine wood.

In addition to the mycorrhiza-producing species MELIN isolated a number of other fungi from mycorrhizal fragments, of which the species of *Penicillium* and *Mucor* are of no particular interest. Of greater interest is a highly virulent fungus, called *M. R. atrovirens* by him, which is exceedingly common and occurs parasitically on roots. One year old sterile plants inoculated with the fungus are at once attacked by hyphae, which penetrate into the epidermal and cortical cells, and the plants die in the course of some few months.

Author's own isolation and synthesis experiments.

Now these experiments (like the experiments of earlier workers) are of little value as compared with MELIN's results, but some of the observations made during my experiments are, however, for various reasons of a certain interest.

To me the principal object was to ascertain whether nitrogen-collecting organisms were found on or in dichotomous mycorrhizae of pine (and of Sitka spruce, which also thrives surprisingly well on dune sand under fairly good climatic conditions), and if so, what is the relation of the root to these organisms.

To ascertain this I made a large number of dispersals from aseptically collected pulverised mycorrhizae from mountain pine, Scots pine, Sitka and Norway spruce as also isolations from mycorrhizae sterilised superficially by sublimate water.

I employed the non-nitrogenous agar, by means of which CH. TERNETZ (1907) isolated nitrogen-fixing fungi from the roots of various Ericaceae.

Among the isolated fungi were two forms which much resembled the *Mycelium radialis atrovirens* described by Melin. They were dominant in all dispersals and isolations from mountain pine in very poor localities.

Both had vigorous dark-green, highly septate hyphae c. $3\ \mu$ thick and filled with oil globules, with no clamp cells, but with a very abundant production of clamydospores as the only fructification observed. A number of hyphae were spiny or granular on the surface and, if so, they were often more colourless.

One of the forms (A) spread very rapidly in the plate with nitrogen-free agar and had a fairly straight growth of hyphae. Its aerial mycelium was grey, later greenish.

The other form (B) grew with very sinuous hyphae, spread more slowly, had an immense greyish-white cushion-shaped aerial mycelium, and fructified somewhat more rapidly and vigorously.

The picture most frequently developed during the dispersals was that the plates were first overgrown by *Penicillium* species, which spread very rapidly, but thinly, over the plate, pushing constantly in front of them a zone in which the agar had grown clear, because the acid given off from the fungi converted the amorphous lime into a beautifully crystallised calcium oxalate. At a later stage some few close-growing dark green colonies of fungi spread over parts of the area passed by the *Penicilliums*, and where it might be assumed that these had already used the diminutive quantities of nitrogen that might have been present as impurities.

By pure cultivation these fungal colonies could always be shown to belong to the two forms A and B described above, and it was therefore natural to assume that they might be capable of assimilating the free nitrogen of the air.

From mycorrhizae of mountain pine, i. a. a form with thin (c. $1\ \mu$) transparent hyphae with exceedingly numerous clamp cells, but without demonstrable fructification, was isolated. Its growth was slow on all substrates, and it formed a dense white cushion-shaped air mycelium. This form (which I will here call C) on account of its appearance was especially expected to be a mycorrhiza producer.

In order to investigate the N-assimilating power of the A and B forms the following experiments were made:

To each form a series of nine Erlenmayer flasks, each with $100\ \text{cm}^3$ Ternetz's nitrogen-free solution, was used. The nine flasks were divided into three sets, all of which were inoculated at the same time.

Set 1 was killed immediately on the addition of 5 cm³ of sublimate water per flask and were set aside as controls.

Set 2 was taken out for Kjeldahl analysis after about two months, and

Set 3 after about three and a half months.

Both species grew exceedingly luxuriantly, at first submerged, but later reaching the surface, where a vigorous air mycelium developed. Their dry weight even amounted to c. 0.5 g per flask.

By the Kjeldahl analysis both the nitrogen content of the fungus itself and that of the nutritive solution left were examined.

For the fungal forms A and B a nitrogen content of a little below and a little over 0.3 per cent, respectively, of the dry weight was found. According to KRUSE (1910) the nitrogen percentage, which e. g. for *Azotobacter* ranges about 10–12 and for the majority of fungi between 6 and 10, may for certain forms decrease to 1–2 or even below 1. Thus 0.3 per cent would seem to be exceptionally low.

In the nutritive solution was found at the start of the experiment 1.8 mg N per 100 cm³, which alone is sufficient to account for the N-content of the fungi. After the experiment the filtrated solutions no doubt contained e. 1.4 mg N per 100 cm³, but some absorption of fixed N from the laboratory air will always take place.

From these facts the conclusion may be drawn that the two fungi are probably not capable of assimilating the free N of the air.

An experiment with a bacterial form which at first grew vigorously on Ternetz agar and which had been isolated from mountain pine from a meagre heath and somewhat resembled *Bacterium radicola* in appearance and growth form, had also a negative result.

While, thus, the microorganisms isolated from the poorest soil were incapable of assimilating the free nitrogen of the air, but on the other hand were very modest in regard to nitrogen, the result was quite different in all the investigated localities presenting relatively favourable conditions for the growth of the mountain pine.

Here many different forms of *Penicillium* and *Citromyces*, a few species of *Phoma*, and many Imperfecti appeared, all of them forms which developed poorly on Ternetz agar. How-

ever, none of the aforementioned A and B forms that required little nitrogen occurred.

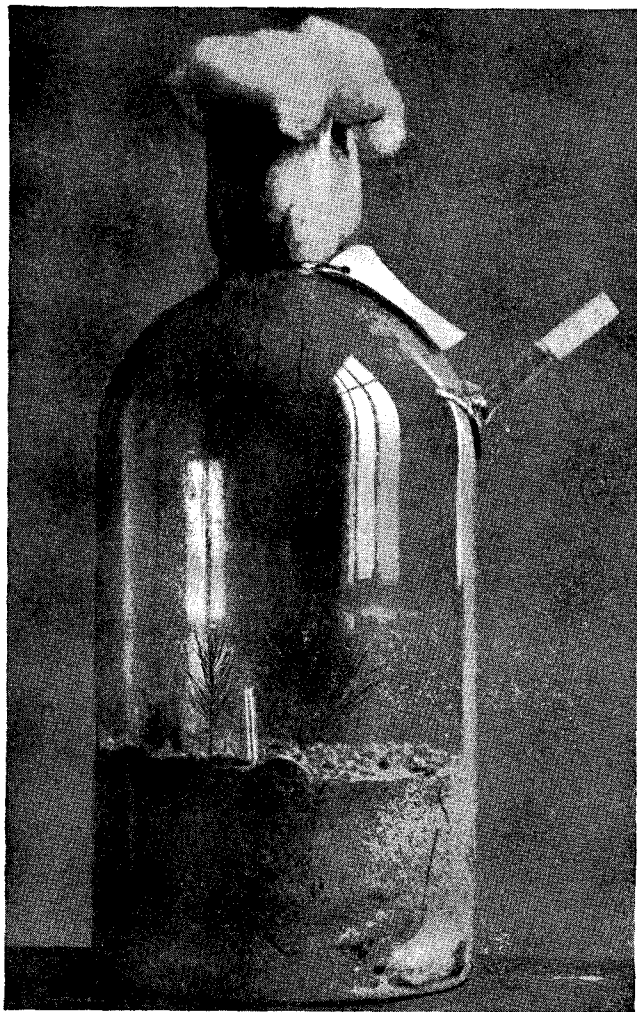


Fig. 5. One year old sterile mountain pines. The glass vessel is 46 cm high.
1-aarige sterile Bjergfyr. Glasset er 46 cm højt.

Authors synthesis experiments

were carried out with ten of the isolated fungi (amongst which the aforementioned forms A, B and C) and one bacterial form in the following way: Sterile plants of mountain pine, Norway spruce, Sitka spruce and European Larch were first produced,

the seeds being shaken for two minutes in absolute alcohol and for one minute in 2 per mille sublimate water; they were washed clean in sterilised water and, while wet, placed for



Fig. 6. Three years old sterile mountain pines. The glass vessel is 46 cm high.
3-aarige sterile Bjergfyr.

germination on sterilised agar in Petri dishes, in which way it became possible to ascertain whether the sterilisation had been effective.

The sterile, just germinated seeds were now sown on sterilised nitrogen-free sand in sterilised glass vessels (46 cm high) with a nutritive solution corresponding to Ternetz agar without sugar; the vessels were closed with cotton-wool, as shown in fig. 5.

With a view to the drainage, pieces of brick were placed at the bottom of the glass vessel, then followed a layer of hygroscopic cotton-wool, and above this the sand. For the sake of ventilation a glass tube was pushed down between the brick fragments.

The watering took place with sterilised water through the spout of the glass vessels by means of sterile apparatus made specially for the purpose and sterilised in a flame before the use.

The sterile plants were inoculated in different combinations immediately on being sown, some vessels being, however, left uninoculated for control.

As a rule three or four seeds were sown in each vessel.

All the plants thrived well during the summer, but the glass vessels had the very obvious disadvantage that they allowed too scanty evaporation. Once wet through, they needed no water for many months. It was not possible, either, to keep them all entirely sterile, but it was possible as to the majority.

The result of the synthesis experiment thus made was negative. When after the lapse of one, respectively three years of growth the experimental plants were taken out, only the A and B fungi of the various fungi used in the experiment had affected the roots, on which they had produced darker thickened areas locally. No *Hartig net* was present, whereas a loose fungal mantle was found, whence numerous hyphae ran without system intracellularly in the outermost cortical cells, but no "digestive process" was observable.

The plants whose roots had been infected in this way were not inferior to the other plants as regards development and appearance.

Although the synthesis experiments had a negative result, they were of interest in another way.

For it was remarkable that some of the plants thrived rather well on the nearly nitrogen-free substrate (nitrogen content 1 mg per 100 g originating from the nutritive solution). For comparison it may be mentioned that the nitrogen content in the poorest places in the Harreskov Sande near Herning (wind-swept sand) was found to vary from 4 to 18 mg per 100 g).

This was especially true of the mountain pines, which at

the end of the third year of growth had attained a height of c. 20 cm and could hardly be contained in the glass vessels.

A determination of the nitrogen content gave on an average c. 7 mg nitrogen per plant, while the average content found by me in 1000 seeds of the quantity sown was 0.27 mg per seed.

Since watering only took place to a very limited extent, the plants must have been able to find and derive benefit from a substantial part of the minimal amounts of nitrogen that were found in the substrate or had possibly been absorbed from the air in the form of NH_3 .

The highly vagrant roots were here probably of advantage to the plant.

That the plants have not assimilated free N from the air, is proved by nutrition experiments. (see below).

Another feature of interest was revealed during the synthesis experiments. Already during the first summer I noticed that one of the vessels was largely filled with water, clear water being seen in several places at the surface of the sand.

In spite of warnings the assistant who was to look after and water the vessels during my absence on official journeys, had "added a little" to this vessel also, which was thus entirely filled with water practically from the start. In the second and third year still more water was occasionally added, so that at last the water level was a couple of centimeters over the sand.

Fig. 6 shows the vessel at the end of the experiment. It will be seen that the development of the three-year old mountain pine plants left nothing to be desired. It is likewise shown how the long monopodially branched roots keep along the wall of the vessel, near the surface and in part running freely in the water.

It is obvious that the addition of oxygen to the roots must have been diminutive. Hardly any other Danish forest tree would have tolerated these conditions. At any rate sterile Norway spruce and larch cultivated in a similar way but without being over-saturated with water, died already after one year. On the other hand, some few Sitka spruces were still alive after the lapse of three years, but their growth had been insignificant. The tallest plants were 6 cm. Plants of Scots pine were not included in the experiment.

IV. NUTRITION EXPERIMENTS

Experiments by other investigators.

A. MÖLLER already made nutrition experiments with one-year old Scots pines and oaks (1903) and with mountain pines (1906) cultivated during one vegetation period in non-nitrogenous sand, which was watered both with and without nitrogen. The experiments had negative results. In the last and most successful of the two experiments (1906) on an average 0.0108 g of nitrogen was found in the one-year old plants of mountain pine before the experiment, 0.0119 g in the two-year old plants developed without nitrogen, and 0.0293 g in the two-year old plants supplied with nitrogen.

From these experiments MÖLLER concluded, probably correctly, that these plants fetched from nurseries and abundantly equipped with mycorrhizae had not been capable of assimilating the nitrogen of the air.

MELIN (1925) has made more extensive nutrition experiments to elucidate the nature of the mycorrhizae.

It appears plainly from these experiments that the mycorrhizae-producing fungi are themselves unable to assimilate the free nitrogen of the air, while, on the other hand, they are quite capable of utilising a number of organic nitrogen compounds, in addition to the inorganic nitrogen compounds, as nitrogen sources.

The relation of the non-mycorrhizal and the synthetically produced mycorrhiza-bearing plant to the above-mentioned nitrogen sources after the experiments appears from the two subjoined tables (l. c. pp. 140 and 144), reprinted here.

It is seen from Table 36 that sterile mycorrhizaless plants cannot absorb the free nitrogen of the air. The experiment, it is true, showed a slight increase of the nitrogen content per plant, but the percentage nitrogen content was decreasing, and in the third year deficiency symptoms occurred, so the increase in the nitrogen content must (with MELIN) be assumed to originate from the fixed nitrogen in the air of the laboratory, which had been taken up by the substrate (e. g. in the form of ammonia).

However, the sterile plants were quite well able to benefit

from the inorganic as well as the organic nitrogen sources. Among the former NH_4Cl was essentially better than KNO_3 .

Among the latter asparagine seemed to be better than nucleic acid and peptone, even quite as good as NH_4Cl . It should be mentioned, however, that the experiment only comprised a total of 23 plants, so we dare not draw highly differentiated conclusions.

It appears from Table 41 that the synthetically produced mycorrhiza-bearing plants are not able, either, to assimilate the free nitrogen of the air. This also applies to mountain pine (Melin 1924). On the other hand they are just as capable as other plants of utilising the inorganic nitrogen sources.

As regards the organic nitrogen sources MELIN i. a. says as follows (1925 p. 79):

“Organische Stickstoffverbindungen. Auf Nukleinsäure und Pepton haben die dreijährigen geimpften Pflänzchen ein kräftigeres und normaleres Aussehen als die nicht geimpften. Die Nadeln der ersteren sind länger und ihre Farbe frischer dunkelgrün als die der letzteren. Bei den Fichtenpflänzchen finden wir ebenso wie bei den Ammoniumpflänzchen eine erhebliche Steigerung der Nadellänge im dritten Jahre. Die Kiefernpflänzchen entwickelten im zweiten Jahre im allgemeinen nur Primärnadeln.

Der Stickstoffgehalt der analysierten Mykorrhizapflänzchen ist grösser als der von Pflänzchen ohne Pilz (Tab. 41). Die grösste Stickstoffmenge finden wir bei einem mit *M. R. silvestris* α geimpften Kiefernpflänzchen auf Nukleinsäure, nämlich 3.25 % der Trockensubstanz und 8.94 mg total, also einen erheblich höheren Stickstoffgehalt als bei den Ammonium- und Asparaginpflänzchen. Die entsprechenden Ziffern für ein normal entwickeltes Pflänzchen ohne Pilz (Fig. 37) sind 1.27 und 2.88. Das Pflänzchen mit Mykorrhiza hat demnach einen ungefähr dreimal so grossen Stickstoffgehalt als das ohne Mykorrhiza. Bei den mit β - und γ -Pilzen geimpften Nukleinsäurepflänzchen ist der Stickstoffgehalt nicht halb so gross als bei dem erwähnten Mykorrhizapflänzchen; er ist aber doch höher als bei dem Pflänzchen ohne Pilz. Die Kiefernpflänzchen auf Pepton verhalten sich ungefähr wie die zuletzt erwähnten Nukleinsäurepflänzchen. Die Fichtenpflänzchen mit Mycorrhiza haben dagegen nur einen unerheblich höheren Stickstoffgehalt als die nicht geimpften Pflänzchen.

Table quoted from Melin 1925 p. 140.

(*Pinus montana* = *P. Mugo*)

Tabelle 36. Stickstoffgehalt analysierter, mykorrhizafreier Pflanzen von *Pinus silvestris*, *P. montana* und *Picea Abies*. Aus Reinkulturen auf verschiedenen N-Quellen.

| Versuch | Alter der Pflanze ¹⁾ | Stickstoffquelle | Gewicht der Pflanze nach Trocknung bei 100° C. g | N-Gehalt ‰ | Gesamtmenge Stickstoff mg | Bemerkungen |
|-------------------------|---------------------------------|--------------------|---|---------------|------------------------------|---|
| <i>Pinus silvestris</i> | | | | | | |
| 1. | 3 | Ohne N | 0.0563 | 0.85 | 0.48 | Fig. 20 Fig. 32 Pflanze abnorm entw. Fig. 21 Fig. 37 |
| 2. | 2 1/2 | » » | 0.0798 | 0.85 | 0.67 | |
| 3. | 2 | » » | 0.0282 | 2.23 | 0.57 | |
| 4. | 3 | KNO ₃ | 0.1645 | 1.66 | 2.73 | |
| 5. | 2 1/2 | NH ₄ Cl | 0.1765 | 2.86 | 5.05 | |
| 6. | 2 1/2 | » | 0.0740 | 2.42 | 1.79 | |
| 7. | 3 | Asparagin | 0.1780 | 3.66 | 6.52 | |
| 8. | 3 | » » ²⁾ | 0.2680 | 3.00 | 8.04 | |
| 9. | 3 | Nukleinsäure | 0.2268 | 1.27 | 2.88 | |
| 10. | 3 | » | 0.2268 | 1.27 | 2.88 | |
| Samen ³⁾ | — | — | 0.00616 | 5.79 | 0.36 | |
| <i>Pinus montana</i> | | | | | | |
| 11. | 2 1/2 | Ohne N | 0.0864 | 0.73 | 0.63 | |
| 12. | 2 1/2 | » » | 0.1126 | 0.75 | 0.84 | |
| 13. | 2 1/2 | » » | 0.0761 | 1.26 | 0.96 | |
| 14. | 2 1/2 | » » | 0.0761 | 1.26 | 0.96 | |
| Samen ³⁾ | — | — | 0.02076 | 5.45 | 0.38 | |
| <i>Picea Abies</i> | | | | | | |
| 15. | 3 | Ohne N | 0.1171 | 0.70 | 0.82 | Fig. 18 Fig. 19, rechts Fig. 19, links Fig. 34 Fig. 39 Fig. 22 |
| 16. | 3 | KNO ₃ | 0.0782 | 3.12 | 2.44 | |
| 17. | 3 | » | 0.0419 | 2.67 | 1.12 | |
| 18. | 2 1/2 | NH ₄ Cl | 0.1719 | 2.82 | 4.85 | |
| 19. | 2 1/2 | » | 0.1624 | 2.69 | 4.37 | |
| 20. | 3 | Nukleinsäure | 0.1322 | 1.51 | 2.0 | |
| 21. | 3 | » | 0.0943 | 1.65 | 1.56 | |
| 22. | 3 | Pepton | 0.1356 | 2.38 | 3.23 | |
| 23. | 3 | » | 0.1356 | 2.38 | 3.23 | |
| Samen ³⁾ | — | — | 0.01922 | 3.44 | 0.22 | |

¹⁾ Anzahl von Vegetationsperioden.

²⁾ Der Sand mit NH₃-bildenden Bakterien fremdinfiziert (Reaktion mit Nessler positiv, mit Diphenylamin-Schwefelsäure negativ; die Versuche mit Wino-gradskys Nährlösung fielen negativ aus).

³⁾ Durchschnittszahl dreier Samen von mittlerer Grösse.

Table quoted from Melin 1925 p. 144.

Tabelle 41. Stickstoffgehalt zwei- bis dreijähriger Pflanzen von *Pinus silvestris* und *Picea Abies* aus Reinkulturen auf anorganischen und organischen N-Verbindungen.

(Mikrogasvolumetrische Bestimmungen nach DUMAS und PREGL) S.54, 76.

| Versuch | N-Quelle | Alter der Pflanze | Mit oder ohne Mykorrhizapilz | Gewicht der Pflanze nach Trocknung bei 100° C. | Stickstoffgehalt | Gesamtmenge Stickstoff | Bemerkungen |
|-------------------------|--------------------|-------------------|------------------------------|--|------------------|------------------------|----------------------|
| | | | | g | ‰ | mg | |
| <i>Pinus silvestris</i> | KNO ₃ | 3 | Ohne Pilz | 0.1645 | 1.66 | 2.73 | Fig. 20 |
| | NH ₄ Cl | 2 1/2 | » » | 0.1765 | 2.86 | 5.05 | Fig. 32 |
| | » | 2 1/2 | » » | 0.0740 | 2.42 | 1.79 | Pflanze abnorm entw. |
| | » | 2 | Boletus luteus | 0.0578 | 2.99 | 1.73 | |
| | » | 2 1/2 | M.R. silvestris γ | 0.1432 | 2.73 | 3.91 | Fig. 33 |
| | Asparagin | 3 | Ohne Pilz | 0.1780 | 3.66 | 6.52 | Fig. 21 |
| | Nukleinsäure | 3 | » » | 0.2268 | 1.27 | 2.88 | Fig. 37 |
| | » | 3 | M.R. silvestris α | 0.2750 | 3.25 | 8.94 | |
| | » | 3 | » β | 0.1490 | 2.56 | 3.81 | Fig. 38 |
| | » | 3 1/2 | » γ | 0.2010 | 1.62 | 3.26 | |
| | Pepton | 3 | » β | 0.1246 | 2.73 | 3.40 | Fig. 41 |
| | » | 3 | » γ | 0.1620 | 2.49 | 4.03 | Fig. 42 |
| <i>Picea Abies</i> | KNO ₃ | 3 | Ohne Pilz | 0.0782 | 3.12 | 2.44 | Fig. 19, rechts |
| | » | 3 | » » | 0.0419 | 2.67 | 1.12 | Fig. 19, links |
| | NH ₄ Cl | 2 1/2 | » » | 0.1719 | 2.82 | 4.85 | Fig. 34 |
| | » | 2 1/2 | » » | 0.1624 | 2.69 | 4.37 | |
| | » | 2 | M.R. Abietis | 0.0739 | 3.13 | 2.31 | Fig. 35 r |
| | » | 2 | » » | 0.0531 | 2.86 | 1.52 | Fig. 35 l |
| | » | 2 1/2 | » » | 0.1131 | 2.58 | 2.92 | Fig. 36 |
| | Nukleinsäure | 3 | Ohne Pilz | 0.1322 | 1.51 | 2.0 | |
| | » | 3 | » » | 0.0943 | 1.65 | 1.56 | Fig. 39 |
| | » | 3 | M.R. Abietis | 0.1170 | 1.86 | 2.18 | Fig. 40 |
| | Pepton | 3 | Ohne Pilz | 0.1356 | 2.38 | 3.23 | Fig. 22 |
| | » | 3 | M.R. Abietis | 0.1632 | 2.23 | 3.64 | |

Diese Versuche zeigen, dass die Mykorrhizen auf komplizierteren organischen Stickstoffverbindungen, beispielsweise Nukleinsäure und Pepton, für die Pflänzchen nützliche Gebilde sind. In Reinkulturen vermitteln nämlich die Mykorrhizen den Pflänzchen die Aufnahme der erwähnten N-Verbindungen im grossen und ganzen leichter, als dies die Wurzel allein tun kann.

Dieses Ergebnis findet auch in der Tatsache eine Stütze, dass die Wurzeln der Mykorrhizapflänzchen nicht übermässig verlängert sind, wie dies bei mykorrhizafreien Pflänzchen auf Nukleinsäure und Pepton der Fall ist. Erstere haben nämlich eine totale Wurzellänge, die derjenigen der Ammoniumpflänzchen nahezu gleichkommt (Tab. 44). Auf Nukleinsäure sind die Wurzeln der Mykorrhizapflänzchen ungefähr 3—3.5 mal kürzer als die der nicht geimpften Pflänzchen."

The conclusions drawn do not seem immediately obvious. If we consider Table 41 separately, it will at once be remarkable that the *uninoculated* three-year plant on asparagine has the highest percentage and the next-highest absolute nitrogen content. Unfortunately it is the only plant on asparagine in the experiment, so a direct comparison with inoculated plants cannot be made. However, according to MELIN's own investigations (l. c. p. 39 and Table 26) asparagine and nucleic acid seem to be of equal value as nitrogen sources for the mycorrhizal fungi.

Taking the means for the two uninoculated pines with asparagine and nucleic acid, respectively, as the nitrogen source, we obtain 2.47 per cent and 5.70 mg of nitrogen and 0.2024 g of dry weight.

For comparison the corresponding means are

for the three inoculated pine plants on nucleic acid
2.48 per cent and 5.34 mg of nitrogen and 0.2083 g
of dry matter — and

for the two inoculated pine plants on peptone 2.61
per cent and 3.72 mg of nitrogen and 0.1433 g of
dry weight.

As regards the three uninoculated and two inoculated plants of Norway spruce on substrates with an organic nitrogen source, on nucleic acid an inconsiderably greater nitrogen content will be found in the inoculated, but on the other hand a slightly greater content of dry matter in the two unin-

oculated plants, as shown by Table 41. On peptone the uninoculated and the inoculated plant will have practically the same contents.

Evidently Table 41 can give no support to Melin's statement quoted above.

Actually it would be more justifiable to use Table 41 as a proof that the mycorrhizal fungi inhibit the uptake by the roots of NH_4Cl .

It will be more correct to interpret Table 36 in conjunction with Table 41 to the effect that the presence or the absence of the fungi does not seem to affect the uptake by the plants of nitrogen from the nitrogen sources used.

Nor do, in my opinion, the photographs of the experimental plants given in MELIN's paper (1925) support his statements.

Apparently figs. 21 and 37 show no poorer vegetative development than figs. 38, 41, and 42. The needles are, indeed, somewhat longer on the inoculated plants (Melin 1925, Table 40) and the roots generally somewhat shorter¹⁾, but judging from the photographs the total picture of growth does not seem more convincing.

Something similar applies to the Norway spruce.

The only valid but not very strong proof lending support to his contention is that the colour is stated to be a more fresh dark-green in the inoculated than in the uninoculated plants. MELIN further says on this subject (l. c. p. 85): "Es sei erwähnt, dass die geimpften Pflänzchen erst in der dritten Vegetationsperiode ein auffallend kräftigeres Aussehen aufweisen als die nicht geimpften Pflänzchen. Es erscheint daher wahrscheinlich, dass sich die Symbiose in den Reinkulturen nicht sofort stabilisiert hat."

However, the possibility is not discussed, viz. that a better colour of the needles and perhaps a higher nitrogen content in the plant (which has not been ascertained) might be due to the liberation of ammonia in the decomposition of the organic nitrogen by the mycorrhizae-producing fungus, without any other

¹⁾ It is peculiar that it is chiefly on nucleic acid that sterile plants of Scots pine and Norway spruce develop long roots, while the development of the root on asparagine and peptone is almost the same in inoculated and in uninoculated plants.

connection with the mycorrhizae than that the fungus happens to act at the same time as an epiphyte.

The results of MELIN's own experiments render such an explanation possible (Melin 1925, Table 26 i. a. showing ammonia production by mycorrhizal fungi in pure cultures on different organic N-sources).

Of course this combination also presents a kind of symbiosis but not the more direct form imagined by MELIN, and very likely not an obligatory form.

In this connection I must mention MCARDLE's experiments (1932), in which synthetic mycorrhizae were produced in pure culture with inorganic as well as with organic nitrogen sources (asparagine, uric acid, glycine, peptone).

According to these experiments there is some probability that while non-mycorrhizal plants in pure culture will thrive on inorganic nitrogen, they show deficiency symptoms on organic nitrogen. However, the presence of mycorrhizae meant no improvement in this respect.

Altogether I think that the conclusions on which MELIN bases his assumption as to an actual mutualistic symbiosis cannot as a whole be said to satisfy the requirements of a scientific proof.

If we compare the results of MELIN's experiments with the extremely distinct results that are obtained with experiments with sterile leguminous plants with root nodules, the uncertainty of the proof as regards the mycorrhizae will be obvious.

Melin (1925, p. 101 et seq.) also mentions the fact that the mycorrhizae of the conifers are especially characteristic of raw humus and often poorly developed on mull as a proof of the positive importance of mycorrhizae to the trees: "Schon hieraus liesse sich der Schluss ziehen, dass die Mycorrhizen in erster Linie für die Stickstoffnahrung der Bäume eine Bedeutung besitzen."

For several reasons, i. a. those given above, such a conclusion can hardly be drawn. Confer also the poor development of mycorrhizae in mor of passive type in opposition to the profuse development, where a nitrification takes place in mor. (p. [17]).

Finally MELIN (l. c. p. 103 et seq.) describes how the fungal mycelia in the raw humus assimilate the complicated

organic nitrogen compounds during liberation of ammonium.

A number of investigations by different authors indicate that by far the greater part of the ammonium occurring in the mor (raw humus) is produced by fungi and is avidly reassimilated by these.

Whether a surplus of ammonia will arise in this way, according to investigations made by WAKSMAN (1916) largely depends on the quantity and the nature of the nitrogen sources available to the fungi. In the case of proteins alone there will be a surplus, since the energy requirement of the fungal organism is greater than its nitrogen requirement. Only part of the nitrogen of the protein molecule will then be utilised in building up the protein of the microorganism. If, however, the energy requirement of the fungi is satisfied by assimilable carbohydrates, only small amounts of ammonia will be liberated.

HESSELMAN (1917 a and b) has studied the capacity of different humus forms to segregate ammonia from a peptone solution. It turned out that mull has a much greater power of segregation than the mor, in which the liberation of ammonia is often so inconsiderable that it can hardly be supposed that a forest should be able to meet its requirements of nitrogen solely by absorbing ammonia.

Also in WAKSMAN's opinion the microorganisms on mor soil are much more dangerous competitors of the higher plants in regard to assimilable nitrogen compounds than on mull, and MELIN regards this fact as a support of his assumption as to the mycorrhizal symbiosis, supposing that the fungi give off part of their nitrogen to the root cells.

It may seem quite probable that this is the case, but in my opinion no proof has been given by these statements. According to MELIN's own investigations sterile roots are just as capable as fungi of assimilating inorganic and certain organic N-compounds, so it is not evident why roots without a fungal symbiosis should be worse off in the competition for the various assimilable nitrogen compounds. The fungi which, without connection with the roots, have broken down the more complex nitrogen compounds, must give off all or part of their booty when they die, being themselves decomposed by bacteria and other microorganisms. Otherwise a mighty accumulation of fungal hyphae in the soil would be observed. And in this way

the roots again and again obtain new possibilities in the competition.

As regards the relation between fungus and root in the mycorrhizae it is natural to ask what is actually known about the way in which nitrogen should be given off by the fungus to the root. Even if we assume, what would seem very probable, that some fluid diffuses from the hyphae into the root cells, it must, on the other hand, be regarded as even more probable that substances pass from the roots into the hyphae. For it is the hyphae which seek out the roots, not the reverse.

In this connection I especially call to mind the phosphatides which are given off by the roots (HANSTEEN CRANNER 1922). MELIN (1925) has himself shown the growth-promoting influence of these phosphatides on the mycorrhizal fungi, and thinks that it is of importance in the formation of the mycorrhizae.

As the phosphatides contain nitrogen, it is not immediately obvious that the interchange of fluids as regards nitrogen is quantitatively to the advantage of the tree plant.

Here we may also mention the investigation by MASUI (1927), who by means of microchemical tests found that the fungi receive from the roots amino acids, carbohydrates, tannin, nitrates, and a number of phosphorus, potassium, and ammonium compounds, while the roots receive nothing in return. His demonstration is based exclusively on a comparison between the contents in root cell and fungus respectively found in microchemical reactions and hence cannot, perhaps, be regarded as final, though it would seem that considerable importance should be attached to it.

The above discussions may, just as well as not, lead to the assumption that the normal ectotrophic mycorrhiza is a mainly epiphytic phenomenon, and that, accordingly, we are not concerned with an actual mutualistic symbiosis, even though the saprophytic treatment of the decaying layer of the forest by the mycorrhizae and other fungi must be regarded as a factor of paramount importance for the growth conditions of the tree plant.

However, especially since 1927 a series of practical experiences and scientific experiments with inoculation soil, or

the like, have been published, some of which, at any rate at first sight, would seem to lend very effective support to the theory of an actual symbiosis.

From Denmark O. PALUDAN (1917), from Australia S. L. KESSEL (1927), from Java J. W. ROELOFFS (1930), and an anonymous paper from Rhodesia (1931, Rhodesian Agr. Journ., quoted from HATCH 1936) have recorded the practical experience that when seed-beds of coniferous plants on agricultural soil or virgin steppe soil failed to thrive so that the plants were stunted, the admixture of inoculation soil from a forest or a nursery which had been working for some length of time may be helpful. At the same time some of the authors observed that the development of mycorrhizae showed a striking improvement, which was, as a rule, taken as a proof that it was the mycorrhiza-producing fungi which the plants had been lacking.

Still a closer inspection of the reports tends to make the problem more complicated.

Most convincing seem to me the reports of KESSEL and ROELOFFS.

KESSEL (1927) describes how the raising of pines on new nursery sites in Western Australia met with difficulties that were not overcome by watering, shading or artificial fertilizers. Failures could not be traced to excessive soil acidity or alkalinity, or to season of sowing, but:

»Experimental work has now shown conclusively that the only method of raising satisfactory planting stock of exotic conifers in Western Australia in a new nursery is first to infect the soil either by applying a light dressing of soil from an old nursery or by transplanting seedling pines from an old nursery and holding them a year in nursery lines.«

ROELOFFS (1930) gives a quite similar report on the difficulties in raising pines on Sumatra. Here the infection of the ground by means of soil from older pine cultures had no satisfactory result, but such were obtained by means of planting older vigorous pine plants in the middle of the transplanting beds with a distance of one yard. Some months afterwards these "middle trees" were surrounded by transplants, which were growing well and showed a green colour strikingly contrasting against the yellowish colour of the rest of the bed. The pictures

given are most convincing and leave to me no doubt that a favourable biological factor has been brought in by the planting of the "middle trees". It seems very probable that this factor may be identical with the mycorrhiza fungi, but the possibility cannot be excluded that the effect is attached to other microorganisms.

Other reports on the same line are however less convincing than the two given above.

O. PALUDAN (1917) describes difficulties with nursery seedlings of conifers on arable ground in Denmark.

But the results are very varying. In one case the species of *Abies*, *Picea* and *Pinus* would not thrive, while *Chamaecyparis* and *Thuja* grew well. In another case *Pinus montana* and *P. silvestris* were normal, whereas *P. Banksiana*, *P. contorta* and *P. Murrayana* sickened.

Inoculation soil from an older conifer nursery (one bag to a bed of undefined size) gave an excellent result, while soil from a 14 year old spruce plantation gave no effect.

H. E. YOUNG (1936) reports on synthetically produced mycorrhizae on *Pinus Caribaea*, *P. taeda* and *P. patula* with *Boletus granulatus*.

He used glass-sided root observation boxes, where sterilized *Pinus* seeds were sown. When a satisfactory root growth was visible, the glass sides were removed and the roots inoculated with isolations from sporophores. Eight weeks later mycorrhizae appeared. There were no controls.

As evidently the applied soil was not sterilized, the reported result of the experiment is not obvious.

At the end of the report the following lines are found:

»In addition to the experiments carried out in the root observation boxes, seedlings of *Pinus caribaea* and *P. patula* growing in pots were inoculated with a culture of *Boletus granulatus* growing on sterilized oats. The growth response was remarkable. The uninoculated pots showed little or no growth and finally died, whilst those inoculated grew vigorously and the foliage became a green healthy colour.

The foliage of the controls was of a purplish red colour. Typical mycorrhiza developed in the inoculated pots.«

Nothing is given about number of pots and plants, weight and nutrient content of inoculation material, length of time, nutrient content of soil, if the seeds and pots were sterilized a. s. o.

To me this report does not seem very convincing. Also it seems extraordinary that all the uninoculated plants died, as most other authors only indicate an inhibited growth as the result of want of mycorrhizae.

A. B. HATCH (1936), produced one-year old pine plants in prairie soil in six flower pots, of which three were uninoculated, three inoculated with a pure culture of mycorrhiza-producing fungi. It is not stated on which substrate this pure culture had been made, and how large a weight was represented by the inoculation material; in my opinion it is absolutely necessary that such information should be given, as I know from my own experiments (see below) that even very small quantities of fixed nitrogen which the substrate may in certain cases absorb from the air (e. g. in the form of ammonia) may be found and very effectively utilised by the experimental plants. An analysis should likewise have been made of the contents, notably of easily available nutrients, in the soil used in the experiments.

The result of the experiment was that the uninoculated plants were appreciably smaller than the inoculated (and mycorrhiza-bearing) plants (405 mg as against 321 mg) and had an extremely low content of K, N, and P. According to the information given the possibility cannot be excluded that these nutrients in a form available to the pine plants were present in such small quantities in the experimental soil that even the small addition represented by the inoculation material may have been of decisive importance for their growth.

This is also suggested by the fact that the plants in one of the three inoculated pots began to turn yellow again in May after having been inoculated in November. This was the case with pot No. 2, which was the first that had showed an increase in growth after the inoculation, but which was thus also the first to stop growth again (and on analysis showed a low percentage content of nutrients). All the plants were harvested at the end of May or the beginning of June, so it was not ascertained whether the needles of the plants in the other pots, also, would have turned yellow that summer.

M. C. RAYNER (1934, 1936, 1939) has taken into consideration the possibility of an addition of nutrients with the inoculation.

In 1930 he made some experiments on very meagre heath soil in Dorset with various species of pine, which were sown either without any addition to the soil or after inoculation of the soil with humus derived from good coniferous woods in Sweden and in southern Ireland. "The experimental plots (area c. 1. m²) were inoculated before sowing by inserting small quantities of the humus material in a series of shallow holes below the seed drills. After filling in the holes, the seeds were sown in shallow drills as in ordinary nursery practice. The amount of humus used was a loosely filled pint measure distributed evenly in 25 holes throughout the yard square plot."

"The immediate result... was improved growth and mycorrhiza-formation by the seedlings, followed in the second season by a rapid falling off in growth and vigour and the conversion of all mycorrhizas into *atrovirens*-*pseudomycorrhizas*".

The result of the experiment, which is not surprising, hardly shows anything, as assumed by the author, as to the importance of the mycorrhizae. In my opinion the experiment only shows that the rather considerable addition of forest humus has caused a better growth of the plants simultaneously with a richer formation of mycorrhiza. It is noted that at any rate one mycorrhiza-forming fungus (*Boletus bovinus*) was found beforehand in the experimental area, so there was a possibility of a natural development of mycorrhizae although such were sparingly present on the one-year old seed-bed plants raised without inoculation soil.

Similar inoculation experiments were later carried out in London on a "compost of light loam and sand with a small amount of leaf humus in the form of leaf mould from broad-leaved trees". Here the good growth continued for two years, or possibly more. The plants in the control plots were a little smaller (but otherwise well developed) and lacked mycorrhizae.

RAYNER (1936) also records some experiments with addition of seven different compost mixtures, i. a. C₁ (1/2 straw, 1/2 dried blood), C₂ (1/2 sawdust, 1/4 birch leaves, 1/4 dried blood), C₅ (hop waste from a brewery + dried blood), C₇ (straw + ammonium sulphate), and C₈ (straw + ammonium phosphate).

"Every treated plot received a dressing of 10 lb compost (moisture content about 75 per cent) per square yard, spread and lightly forked in before sowing".

C₅ was the best, then came C₂, C₁ and C₇, all of which gave a very strong positive result. The remaining plots yielded lower results, though their plants were larger than those of the control plot (C₀). The development of mycorrhizae was most profuse where the plants were largest.

In order to meet the objection that the results were solely due to the considerable dressing applied with the compost, RAYNER (1939) records the following investigations:

The analyses made revealed an especially high absolute content of nitrogen and P₂O₅ in composts C₅ and C₁, viz. in per cent:

| | C ₅ | C ₁ |
|-------------------------------|----------------|----------------|
| N | 4.64 | 3.33 |
| P ₂ O ₅ | 2.80 | 0.77 |

In addition the content of directly available nutrients was determined and experiments were made with as well as without any addition of the composts but with addition of inorganic nutrients in the same quantity as the content of directly available nutrients found in the composts.

The compost yielded about twice as large an increment as the inorganic nutrients, which, however, gave a good result as compared with the control plot. The proportions were e. g. as follows:

| | | | |
|----------|----------------------|----------|------|
| Series 1 | control | weight g | 15.7 |
| — 2 | C ₅ | | 65.3 |
| — 3 | C ₅ salts | | 43.1 |
| — 4 | C ₁ | | 85.3 |
| — 5 | C ₁ salts | | 25.7 |

The author i. a. draws the following conclusions:

"The results confirm conclusions previously reached in respect to the existence of actively deleterious substance in the experimental soil. They show further that addition of organic composts put an end to the production of such substances, whereas addition of equivalent amounts of available nutrients as inorganic salts is practically without effect."

In my opinion the author in his conclusions goes beyond what can be safely maintained.

The better effect of the compost must probably be explained by the fact that biologic processes during the growth of the plants constantly make fresh amounts of nutrients directly available.

MITCHELL, FINN and ROSENDAHL (1937) experimented with 1—2 year plants of *Pinus strobus* and *Picea rubra*. "The soil used in the seed beds consisted of a mixture of unwashed sand, thoroughly composted sawdust and a small amount of clay... which was supposed to have physical properties approximating those of sandy nursery soils, but... so infertile chemically that seedlings grown therein respond readily to fertilizer applications".

Much care was taken to ensure that the physical conditions should be quite uniform in all the beds, and all the beds with the exception of certain controls (which did not get fertilizer either) were inoculated with 50 g soil known to be rich in mycorrhiza-forming fungi. The size of the beds is not indicated. All the beds except the controls got fertilizers in different quantities.

During the second year mycorrhizae appeared also in the uninoculated control beds but only on such groups of plants as had started to grow more vigorously. Good growth and formation of mycorrhizae always seemed to coincide. While all the pine plants were growing, although at two very different rates, only the mycorrhiza-bearing spruce plants survived the second growing season.

The experiments with inoculated and fertilized beds gave the following results:

"that seedling harvested during the second growing season from infected areas of the poorest soil used — — — (control beds) — — — had the best developed mycorrhizae and the greatest proportion of mycorrhizal short roots.

Those grown in soils of intermediate fertility possessed fewer and less developed mycorrhizae. And mycorrhizae were infrequent, poorly developed or entirely lacking... in all beds supplied sufficient fertilizer to preclude any possibility of nutrient deficiency." These heavily fertilized seedlings were by far the biggest of the lot.

In order to test the hypothesis advanced by BURGESS (1936), according to which the beneficial effect of the mycorrhizae (if any) is due to the mineralising activity of the mycelium in the sub-

strate, chemical analyses were made of soil samples taken from infected and uninfected areas of the control beds. Only insignificant differences were found.

As it might be objected that continuous removal by seedlings growing in infected areas would tend to prevent any measurable accumulation of nutrients liberated by the fungi, "tests were also made of soil samples from infected and uninfected areas in which, due to removal the previous year, no seedlings were growing at the time. The results of these analyses were essentially the same".

The authors come to the same conclusion as HATCH (1937) that the mycorrhizae "are of benefit to coniferous seedlings growing in . . . all but the most fertile or artificially maintained soils, and that seedlings, lacking mycorrhizae, are unable to exist in very infertile substrates".

Although their description of the appearance of the mycorrhizae in the control beds may sound very convincing, still it leaves doubts.

The authors only see one factor which can be supposed to have varied in the substrate, namely the formation of mycorrhiza, and hence draw their conclusion.

And still the thought can in no way be considered unlikely, that also other biological factors may have varied with the same result, for instance the decomposition of sawdust caused by fungi or bacteria or both. Further the fixation of free N from the air by fungi or bacteria or both, etc.

It can even be considered as most likely that such processes have really started in the newly mixed soil, and that they will start and spread in a manner much like that described by the authors for the mycorrhiza fungi.

The authors' chemical analyses of soil samples mentioned above do not in my opinion prove that no such biological activities have taken place, it being most likely that liberated nutrients will have been absorbed possibly through diffusion by the plants neighbouring to be examined empty "spots" of the beds, or have been washed out.

FRANK J. MILLER (1938) records some experiments made in an American nursery with 1/0 shortleaf pine on soil which had the same spring received 2 cubic yards per acre of a

"compost, containing pine & hardwood litter and humus treated with commercial fertilizer". In three "test-plots" there had during the two previous years been 1) shortleaf pine - cow pea, 2) shortleaf pine - hardwood, and 3) shortleaf pine - shortleaf pine transplants, respectively.

In case 3) the length of the primary shoot during the first year was more than twice as large as in cases 1) and 2), and similarly the mycorrhizal development was very prolific while in cases 1) and 2) it was very sparse.

The author sees the explanation in the fact that in case 3) pines had been cultivated in the area the year before the experiment. In my opinion the result of the experiment seems peculiar since all the plots had received a supply of inoculation soil shortly before (through the compost), and all of them had borne seed-bed plants of pine two years previously.

The author also mentions another experiment, in which "new soil was inoculated with soil and roots taken from thriving shortleaf pine areas. These plots did not show an increased growth of shortleaf pine seedlings over those in check plots. However the plots will be kept under observation..."

DONALD P. WHITE (1941) showed by green house trials, that the early development of *Fraxinus pensylvanica*, *Ulmus americana* and White pine was much better in forest soil than in prairie soil with the same content of nutrients.

He saw the explanation in the presence, respectively need of mycorrhizae and tried to give a proof in the following way:

Sterilized seeds of white pine were sown in jars with a) pure prairie soil and b) prairie soil with 10% of humus top soil from the Wisconsin Rapids state nursery. The growth in case b) was twice the growth in case a, and the plants in b) showed mycorrhizae, in a) not.

Further a pure culture experiment was performed. Mycorrhizae were cut off from the roots, sterilized and forced beneath the surface of nutrient agar in petri dishes. The mycelium emerged was several times transferred and finally inoculated on sterile cornmeal-sand medium. It is described as a fine, septate, white to yellowish mycelium, but clamp cells are not mentioned.

The rest of the experiment is reported as follows:

»Cultures in Erlenmeyer flasks were prepared as described by MELIN (1922) and planted aseptically with two sterilized, germinated seeds of red pine. After 20 days some flasks showed contamination and were discarded. Half of the flasks were then inoculated with about one gram of cornmeal-sand inoculum. The others were retained as checks. The flasks were left in the greenhouse for three months. At the end of this time half of the uninoculated plants were alive. The inoculated plants had large crowns and considerably better root development with many swollen root apices (fig. 7)«

It is not mentioned whether mycorrhizae were formed or not, and from fig. 7 it can't be seen. It may therefore be assumed that no mycorrhizae were found. It seems unlikely that real mycorrhizae should have been stated and the fact not mentioned at all.

The fact, that the inoculated plants were better, may satisfactorily be explained by the addition to every two seedlings of 1 gram cornmeal-sand which during a month had been substratum for a fungus. The quantities of ammonium hereby formed (cf. Melin 1925) may have been sufficient to account for the differences noted.

The result of the experiment in jars with prairie soil, forest- or nursery soil and mixtures respectively may be explained by differences in the biological processes outside the root as well as in the physical conditions on which we are not informed. These factors may considerably influence the root activity as well as the biological processes in the soil. If the evaporation from the plants has been by far the greatest on forest soil, this fact alone would be enough to account for the differences, as the assimilation is dependant on the time in which the stomatas have been kept open in light.

If we study the experiences and experiments thus described, it seems to me that the following conclusions can be drawn:

Practical experiments in Australia, Sumatra and perhaps other places have shown that under certain conditions seedlings and transplants of pines on soils that had never borne conifers before would not thrive, until a treatment with inoculation soil or a planting of older vigorous pines as "middle trees" in the beds was performed.

Although experiences do not quite agree (on Sumatra inoculation soil had no satisfactory effect) still it can hardly be doubted that by the application of inoculation soil or planting of older "middle trees" a favourable biological factor has been brought into action in these cases. It seems very probable that this factor should be the forming of mycorrhizae, but no real proof has hitherto been given, and the possibility can not be excluded that it may be some biological factor acting outside the roots.

A series of more scientifically formed experiments of later date on the question are reported in literature. But the authors are generally somewhat hasty in drawing their conclusions, neglecting to investigate the possibilities of other explanations that might be conceivable.

Even if the experiments with prairie soil made by HATCH (1937) might be considered as true manifestations of a strong effect of a purely biological nature associated with the mycorrhiza-producing fungi, it is still not certain that we are concerned with an actual symbiosis as this word is generally understood. It may quite possibly be an effect exclusively associated with the ordinary decomposition by the fungi of plant remains and humus substances.

The only way in which to obtain a definite result is that of pure cultures, like MELIN'S but on a much larger scale, and very detailed chemical and physical analyses.

It may finally be mentioned, that the practical experience in the establishment of new nurseries in general is that there is no difficulty about raising vigorous young deciduous or coniferous plants on former arable land or heath, or coniferous plants on former deciduous forest soil.

Causes of the formation of mycorrhizae.

Among the papers published during recent years there are some which attempt a more detailed exposition of the *actual causes of the formation of mycorrhizae*.

The results of MITCHELL, FINN and ROSENDAHL in this respect were reported above and showed that in the inoculated beds the development of mycorrhizae was only abundant if one of the three substances N, K, or P were not added, and mycorrhizae failed to appear when all three substances were abundantly present.

HATCH (1937) has made a number of pot experiments with the result that the pine formed mycorrhizae when a relative deficiency of N, P, K, and Ca, or one of these substances, prevailed in the plant, but check experiments performed by BJØRKMANN (1942) seem to show that it is no *internal* deficiency in the plants which conditions the formation of mycorrhizae.

BJØRKMANN (1942) has made very comprehensive culture experiments in order to elucidate the conditions for the formation of mycorrhizae in pine and Norway spruce. He arrives at the result that the determining external factors are especially the illumination and the supply of soluble nitrogen and phosphoric compounds.

The formation of mycorrhizae requires a certain intensity of light, which is higher for pine than for spruce. In general the occurrence of mycorrhizae at a light intensity of c. 25 per cent of full light is of approximately the same extent as at higher light intensities. In soils with a lively nitrogen mobilisation the greatest amount of light is required for an optimal development of mycorrhizae. If the light intensity is below 25 per cent, the frequency decreases rapidly, and at less than 6 per cent light, no mycorrhizae have been observed.

As regards N and P, conditions are less clear. BJØRKMANN, however, thinks that the following main features can be outlined.

A marked lack of nitrogen or phosphorus, or both, in the substrate will inhibit the formation of mycorrhizae, and a very high simultaneous content of assimilated nitrogen and phosphorus will exclude it. With a high content of assimilable nitrogen but a low content of phosphorus or a high content of phosphorus and a low content of assimilable nitrogen, however, mycorrhizae may develop luxuriantly.

“Die Mykorrhiza entwickelt sich also optimal, wenn unter im übrigen günstigen Bedingungen (insbesondere genügendem Licht) im starker (jedoch nicht völliger) Mangel entweder an löslichem Stickstoff oder an Phosphorsäure im Substrat herrscht”.

BJØRKMANN likewise explains the influence of light, nitrogen, and phosphorus on the development of mycorrhizae as a result of the influence of these factors on the contents of soluble carbohydrates in the root cells.

“Bei einer hohen Lichtstärke geht eine lebhaftere Kohlenhydratproduktion vor sich, wodurch den Mykorrhizapilzen eine grössere Menge Zucker zugänglich wird. Wenn entweder ein starker Mangel an löslichen Stickstoff oder an Phosfor im Substrat vorliegt, wird die Eiweissynthese gehemmt, und es entsteht ein Überschuss an löslichen Kohlenhydraten in den Wurzeln der Wirtspflanze, wobei sich Mykorrhiza bildet. Kommen sowohl leichtzugänglicher Stickstoff wie Phosfor in reichlicher Menge im Substrat vor, so entsteht dagegen kein Überschuss an löslichen Kohlenhydraten in dem in diesem Fall kräftig heranwachsenden Pflanzen, weshalb Mykorrhizabildung ausbleibt. Dasselbe dürfte auch bei spärlicher Beleuchtung der Fall sein. . . .”

BJØRKMANN'S result is an example of a well-conceived hypothesis; however, in this case, also, no proof of the correctness of the hypothesis has been submitted. First of all it must be noted that from the start the mutualistic symbiosis is taken for granted, the object being then to find a satisfactory explanation of it. No other possibilities have been glanced at. And yet the explanation does not seem too remote that the mycorrhiza-producing fungi only seek the roots on account of the phosphatides and possibly other secretions which they only need when the substrate is relatively poor in nitrogen or phosphorus, and which the roots do not give off in noteworthy quantities under very poor growth conditions.

In my opinion all the features observed by BJØRKMANN (as well as by MITCHELL, FINN and ROSENDAHL) may be satisfactorily explained from this point of view.

It can, for instance, be mentioned that on pronounced mull soils the majority of mycorrhiza-producing fungi (on conifers) are absent or only sporadically present.

The symbiosis theory will explain this as due to the circumstance that on mull soil a somewhat lower percentage of soluble carbohydrates is found in the root cells.

But would it not be just as natural an explanation that the appropriate fungi are absent or rare because under the circumstances it is chiefly bacteria (and some fungi which do not form mycorrhizae on conifers) which decompose the litter.

Of interest in this connection is also KÜRBIS'S observation (1937) that the number of fungal spores and bacteria in the

soil increases considerably towards the roots, even quite young roots. This suggests that what attracts the fungi is secretions from the root (e. g. of phosphatides, cf. above), possibly in connection with knocked off parts of the root, and not the content of the living root cells of carbohydrates in solution.

Finally it must be mentioned that

LINDQUIST (1939) has found that mycorrhiza-forming fungi in pure culture left certain matters in the substrate which acted as growth promoters on young spruce plants later cultivated in the same substrate (after cleaning through a Berkefeld filter), but no calculation of the standard error of the experiment was made. Such calculations combined with a critical revision of the applied methods in my opinion give the result that no other conclusions can be safely drawn, than that certain parasitic fungi probably leave growth restraining matters in the substratum. LINDQUIST proposes an exchange of growth substances as the cause of the establishment of a mutualistic symbiosis.

Symbiosis or not?

On p. [34] and [35] we already started the discussion on this topic but left it for further examination of existing reports, yet touching it now and again afterwards.

If on the basis of a more direct estimate we try to answer the question: Symbiosis or no symbiosis?, notably the nearly complete lack of root hairs of the mycorrhiza will speak in favour of the symbiosis, while the great number of fungi which are evidently capable of forming mycorrhizae on the same species of tree will speak against it.

On the one hand, the idea naturally suggests itself that the hyphae radiating from the root possibly replace the root hairs and accordingly are beneficial to the tree plant.

On the other hand, it seems little probable that a so finely balanced state as a genuine mutualistic symbiosis with the root may be brought about by a great many different fungal species on the same species of tree.

In support of the supposition of the fungal hyphae functioning as root hairs it has, as a rule, been urged that the dense and often thick fungal mantle which surrounds the root hardly permits the passage of water in any other way than

with the cooperation of the fungal hyphae, so that the water has to pass through the hyphae to reach the root cells. However, this argument hardly holds good. It is true that the hyphae in the fungal mantle form a pseudoparenchymatic tissue. But it is, indeed, only pseudoparenchymatic. If we look at a good microscopic picture (as e. g. my fig. 1), the considerable variation in size and shape of the surfaces restricted by the hyphae walls will be obvious. It seems improbable that they should all represent cell lumina.

Everything indicates that the hyphae grow quite casually among each other with no other plan than trying to approach the root. Hence it is most natural to assume that a fairly dense tissue arises in this way, but that this will contain such a great number of irregularly disposed open pores that there will be ample opportunity for the passage of water.

It is even conceivable that the tissue owing to the capillary action may acquire a water-absorbing capacity similar to that of a sponge.

In the same way it may be assumed that the bundles of hyphae radiating from the root may by a capillary action convey water nearer to the root. For it is, as a rule, bundles of hyphae (rhizomorphs), not so often individual hyphae, which are seen to radiate from the root.

If actually the hyphae are substitutes for the root hairs, it need not necessarily be in the way that they convey the soil water through their lumina.

In this connection it is interesting that A. B. HATCH (1937), treating the question of the total absorbing surface of roots of equally large plants with and without mycorrhizae, respectively, arrived at the view that in spite of their shorter root system the absorption surface is absolutely largest in the mycorrhiza-bearing plants thanks to the more extensive ramification and the greater thickness of the short-roots as well as the retarding influence of the fungal mantle on the suberisation of the endodermis and cortex, and finally on account of the radiating hyphae.

In fact, a new theory concerning a beneficial effect of the fungi might be advanced, based on a motion of the soil water between the hyphae instead of through them, which makes it easier to explain that so many different species of fungi may produce mycorrhizae on the same species of tree.

But this theory cannot be based on solid proofs any more than the aforementioned theories.

The correct attitude to be taken up to-day to the whole problem is quite evidently an expectant attitude.

The thought of an actual symbiosis may perhaps seem the most probable but, as appears from the above, it has not yet been based upon proofs.

The possibility must therefore still remain open, though evidently less attractive to human imagination than the mutualistic symbiosis, that the intimate association is only of positive benefit to the fungi.

Author's own nutrition experiments.

Of my own nutrition experiments only the subjoined extracts are of interest at present:

The main point to me was to elucidate the importance of the mycorrhizae for the nitrogen assimilation, more especially the question as to whether the mycorrhizae of the pines were capable of absorbing the free nitrogen of the air.

Although A. MØLLER's and MELIN's experiments, summarised above, had given negative results, the question could not be regarded as definitely answered.

MELIN's experiments were made under laboratory conditions with aseptically produced synthetic mycorrhizae, and the possibility could not be excluded that mycorrhizae produced by other fungi, possibly in connection with bacteria under natural conditions, might fix the free nitrogen of the air.

In A. MØLLER's experiments only one-year old nursery plants of mountain pine were used during one growth season, and it might perhaps be imagined that conditions would be somewhat different with a little older plants from a site poor in nitrogen cultivated in experiments for several years. And A. MØLLER's experimental result seemed, indeed, to have a few assailable points.

His material consisted of too small a number of plants, viz. six one-year old plants without nitrogen and seven with nitrogen, respectively, and the mean weight of the two groups at the start of the experiment is not stated.

In addition, the plants cultivated without nitrogen also

showed a small increase of nitrogen, and to me it was of essential importance that no plants of mountain pine were included in the experiment.

Finally it might be supposed that a possible fixation of the free nitrogen of the air dependent on the presence of the pine plants was effected by microorganisms which did not participate directly in the formation of mycorrhizae but took part of their nourishment from the sloughed off dead parts of the roots of the mountain pine, which are very abundant especially in meagre soil. As will be known, SAIDA (1901), TERNETZ (1907), FRÖLICH (1908), STAHEL (1911), and other workers are of opinion that they have ascertained the fixation of the free nitrogen of the air by fungi, i. a. moulds, and in addition it appears that the nitrogen assimilation was greatest when some fixed nitrogen was present. Notably FRÖLICH (1908) found nitrogen-fixing Hyphomycetes on dead plants.

I therefore made the following nutrition experiments:

a) The nitrogen content was determined of 1000 mountain pine seeds capable of germinating, and was found to average 0.27 mg per seed.

Twenty seeds of this average weight were sown unsterilised in two flower pots with sand heated at 800° at the Royal Porcelain Factory and in a subsequent analysis found to be free of nitrogen. In this as in all the other nitrogen determinations referred to in the present work A. C. ANDERSEN'S and NORMAN JENSEN'S (1925) modification of Kjeldahl's method was employed.

To the sand had been added 2 g tribasic calcium phosphate per kg, and it was watered with a solution of 1.5 g Mg SO_4 , 2.5 g K H S O_4 , a knife-point full of Na Cl , and 10 cm^3 Fe Cl_3 (10 %) to 100 litres distilled water.

The pots were covered with tight-fitting reversed glass vessels and placed in the open, but sheltered from rain by a glass roof.

All the seeds germinated and all developed non-mycorrhizal roots, so the mycorrhizal fungus does not seem to accompany the seed.

At the end of the season (May-October) one pot had become infected with Chlorophyceae and was eliminated. As to the other pot, the average fresh weight of the plants was 48 mg

and their average nitrogen content 0.29 mg. The needles were light yellowish at the tip. The sand was found to be free from nitrogen after the culture.

b) Two years or several years old self-sown mountain pines from the poorest possible site (wind-swept almost vegetationless patches in the Harreskov Sande near Herning in Jutland) were cultivated in sand culture with and without nitrogen.

The plants were placed singly in pots. Finely ground and heated quartz obtained from the Royal Porcelain Factory and found by the Kjeldahl analysis to be free of nitrogen (just like some examined pot sherds) were used as culture soil. The pots were covered with cylindrical glass vessels turned upside down and fitting closely to the edge of the pots without being absolutely tight.

The experiment was not aseptic, and the glass vessels were only meant as a protection against insects, dust, etc. The pots were placed in the open, but were sheltered from down-pour by a glass roof.

They were watered with a solution of 1.5 g magnesium phosphate and 1.5 g potassium bisulphate to 100 litres of water, for some of the plants with an addition of 10 g of ammonium nitrate, but for most of them without any addition. Beforehand the sand had been found to contain Ca, Na, and Fe in sufficient and available quantities.

The reaction of the sand was neutral to slightly alkaline, but the nutritive solution was made faintly acid (orange to red with methylene red) by the addition of hydrochloric acid.

In the experiments plants with markedly dichotomous and markedly racemose mycorrhizae were kept separate.

Before planting each plant was described and the fresh weight determined after the roots had been cleaned of sand and quickly dried with blotting paper.

A number of plants corresponding to the number planted were selected for nitrogen determination; they were arranged according to size, and the result was as follows (Table 1):

By means of Table 1 the nitrogen content at the start of the experiment was determined for the plants included in the experiment. After two growth periods the plants were taken out and their nitrogen contents determined. The result of the experiment appears from Table 2.

Table 1. 1st Experiment.
Nitrogen content in mountain pine plants from Harreskov Sande.
Indhold af Kvælstof i Bjergfyrplanter fra Harreskov Sande.

| Type and number of the plants <i>Planternes Type og Antal</i> | Fresh weight <i>Friskvægt</i> | | Nitrogen content <i>Indhold af Kvælstof</i> | | |
|---|----------------------------------|-------------------------------|--|-------------------------------|---|
| | total ialt mg | per plant pr. Plante mg | total ialt mg | per plant pr. Plante mg | per g fresh weight pr. g Frisk- vægt mg |
| 3 one-year plants with some few dichotomies..... 3 eenaarige med enkelte Dichotomier | 135 | 45 | 0.90 | 0.30 | 6.7 |
| 2 one-year plants without dichotomies 2 eenaarige uden Dichotomier | 88 | 44 | 0.64 | 0.32 | 7.3 |
| 14 dichotomous 2-4- year plants..... 14 Dich. 2-4 aarige | 1235 | 88 | 4.51 | 0.32 | 3.6 |
| 12 racemose (i. e. without dich.) 2-5-year plants.... 12 racemøse 2-5 aarige | 1630 | 136 | 4.48 | 0.38 | 2.7 |

Table 2. 1st Experiment
with self-sown mountain pine plants from Harreskov Sande
cultivated for two years on N-free sand culture
Nitrogen content in mg
Kvælstofindholdet i mg

| Type and number of the plants <i>Planternes Type og Antal</i> | At planting <i>Ved Plantningen</i> | | After the 2nd year of growth <i>Efter 2. Vækstaar</i> | |
|--|---------------------------------------|-------------------------|---|-------------------------|
| | total ialt | per plant pr. Plante | total ialt | per plant pr. Plante |
| <i>With addition of nitrogen</i> <i>Med Kvælstof-Tilskud:</i> | | | | |
| 2 dichotomous 2-year plants (dichotomy faintly developed) 2 dichotome 2-aarige (Dichotomien kun svag) | 0.9 | 0.45 | 3.8 | 1.90 |
| 5 racemose 2-year plants..... 5 racemøse 2-aarige | 2.1 | 0.42 | 7.8 | 1.56 |
| <i>Without addition of nitrogen</i> <i>Uden Kvælstof-Tilskud:</i> | | | | |
| 8 dichotomous 2-year plants.. 8 dichotome 2-aarige | 4.0 | 0.50 | 4.8 | 0.60 |
| 6 racemose 2-year plants..... 6 racemøse 2-aarige | 3.2 | 0.53 | 3.9 | 0.65 |
| 1 dichotomous 2-year plant.. 2 dichotom 2-aarig | 0.5 | 0.50 | 0.9 | 0.90 |

In all the pots a fairly abundant vegetation of algæ, chiefly *Chlorophyceae*, had gradually appeared on the surface of the sand, even the first summer. At the end of the experiment this algal layer and the immediately subjacent sand were peeled off

Table 3. *1st Experiment.*

Nitrogen content after the experiment in the different layers of the sand used.

Kvælstofindholdet efter Forsøget i de forskellige Lag af det benyttede Sand.

| Number and type of the plants in the pots <i>Antal og Type af Potternes Planter</i> | Algal layer with subjacent sand <i>Algelaget med underliggende Sand</i> | | Remainder of the sand <i>Resten af Sandet</i> | | |
|--|--|-------------------------------|--|---|--|
| | Dry weight <i>Tørvægt</i> | N-content <i>N-Indhold</i> | Total dry weight <i>Samlet Tørvægt</i> | Number of samples of 100 g <i>Antal Prøver á 100 g</i> | average N-content of 100 g <i>Gennemsnitlig N-Indh. pr. 100 g</i> |
| | g | mg | g | | mg |
| 8 dichotom. 2-year plants <i>8 Dichot. 2-aarige</i> | 116 | 71.4 | 2855 | 4 | 1.6 |
| 6 racemose plants <i>6 racemøse 2-aarige</i> | 164 | 27.2 | 1747 | 3 | 1.3 |
| 1 dichot. 2-year plant . . . <i>1 Dichot. 2-aarig</i> | 117 | 32.8 | 867 | 1 | 2.5 |
| Check pot without plants <i>Kontrolurtepotten uden Planter</i> | 78 | 26.4 | 899 | 2 | 1.3 |

all the pots and its nitrogen content determined separately. Of the remaining greater part of the sand a suitable number of samples of 100 g of dry weight each were taken out and their nitrogen content likewise determined (see Table 3).

The fairly high nitrogen contents thus found were surprising, as the watering had been done very carefully. They must give rise to the conjecture that a fixation of the free nitrogen of the air had taken place in the algal layer. Since at the same time the development of the experimental plants had not been really prolific, possibly on account of the liability of the sand to form a kind of hard crust, the experiment was repeated under extended and safer conditions.

c) This time, also, plants from Harreskov Sande were used.

When the experiment was commenced, all the plants were described, weighed, and divided, according to estimate, into three

Table 4. 2nd Experiment.
Nitrogen content in mountain pine plants from Harreskov Sande
analysed at the start of the experiment.

Indhold af Kvælstof i Bjergfyrplanter fra Harreskov Sande.

| Number of plants <i>Planter Antal</i> | With dichotomous mycorrhizae <i>Heraf med dichotome Mykorrhizer</i> | Fresh weight <i>Friskvægt</i> | | Nitrogen content <i>Indhold af Kvælstof</i> | | |
|---|--|----------------------------------|--------------------------------------|--|--------------------------------------|---|
| | | total <i>ialt</i> mg | per plant <i>pr. Plante</i> mg | total <i>ialt</i> mg | per plant <i>pr. Plante</i> mg | per g fresh weight <i>pr. g Frisk- vægt</i> mg |
| S 5 | 2 | 620 | 124 | 2.1 | 0.4 | 3.3 |
| S 5 | 0 | 336 | 67 | 1.2 | 0.2 | 3.6 |
| S 5 | 2 | 980 | 196 | 3.2 | 0.6 | 3.3 |
| S 5 | 3 | 520 | 104 | 1.3 | 0.3 | 2.5 |
| S 5 | 1 | 605 | 121 | 1.6 | 0.3 | 2.7 |
| S (means of 25 plants). <i>S (Middeltal af 25 Ptt.)</i> | | | 122 | 9.4 | 0.4 | 3.1 |
| M 5 | 3 | 1240 | 248 | 4.2 | 0.9 | 3.4 |
| M 5 | 4 | 1295 | 259 | 3.1 | 0.6 | 2.4 |
| M 5 | 0 | 1050 | 210 | 3.1 | 0.6 | 3.0 |
| M 5 | 5 | 1350 | 270 | 4.1 | 0.8 | 3.0 |
| M 5 | 3 | 1315 | 263 | 3.5 | 0.7 | 2.6 |
| M (means of 25 plants). <i>M (Middeltal af 25 Ptt.)</i> | | | 250 | 18.0 | 0.7 | 2.9 |
| L 5 | 4 | 3600 | 720 | 9.6 | 1.9 | 2.7 |
| L 5 | 5 | 3675 | 735 | 11.7 | 2.3 | 3.2 |
| L 5 | 3 | 2515 | 503 | 7.3 | 1.5 | 2.9 |
| L (means of 15 plants). <i>L (Middeltal af 15 Ptt.)</i> | | | 653 | 28.6 | 1.9 | 2.9 |

Mean of all the groups 3.0 ± 0.38

S = small plants; M = medium-sized plants; L = large plants.

classes of magnitude: large, small, and medium-sized (later designated L, S, and M). At the start of the experiment sixty-five plants were taken out for nitrogen determination, divided into groups of five. The result will be seen from Table 4.

If we compare Tables 1 and 4, it will be seen that one-year old plants have a somewhat higher percentage content of nitrogen than the somewhat older plants. However, from about two years and upwards the percentage content of nitrogen seems to be fairly constant. (The largest plants were c. 10 years old).

The mean error in the determination of the nitrogen content in a group of five plants would be c. 13 per cent and in a determination of the nitrogen content of all the 65 plants c. 4 per cent.

In addition, the two tables show that a more or less widespread occurrence of dichotomies is of no importance for the percentage content of nitrogen. On the other hand the largest plants seem to have the largest number of dichotomies; however, this can hardly be explained by the supposition that the size depends on the dichotomies (cf. p. [18]).

Finally it is remarkable that these plants from a poor site have such a low percentage content of nitrogen, viz. only 3.0 mg nitrogen per g of fresh weight. For comparison it may be mentioned that the plants cultivated by A. MÖLLER (1906) with an addition of nitrogen contained 6–10 mg of nitrogen per g of fresh weight (the weight of the dry matter according to KÖNIG (1906) being fixed at 40 per cent of the fresh weight), while his plants cultivated without nitrogen after the experiment only contained 2.7 mg per g.

The plants included in the cultivation experiment were treated in the following way:

The individual plants were at once numbered S_1 , S_2 , etc. M_1 , M_2 , etc., and after weighing and description they were planted *each in its* numbered unglazed pot containing c. 300 cm³ (0.42 kg) of heated nitrogen-free sand to which 2 g of tribasic calcium phosphate per kg had been added. Subsequently it was watered with a solution of 1.5 g Mg SO₄, 3.5 g K H S O₄, a knifepointful of Na Cl, and 10 cm₃ Fe Cl₃ (10 per cent) — all in 100 litres of distilled water. All the chemicals used, the sand, the potsherds, and the distilled water, had been examined beforehand and found to be free of nitrogen. For the distilled water must be noted, however, that even though qualitative reactions which show quantities in solutions right down to less than $\frac{1}{1\,000\,000}$ (e. g. CIMMINO's hydrochloric acid-diphenylamin sulphuric acid

Table 5.
with self-sown mountain pine plants from Harreskov Sande

Forsøg med selvsaaede Bjergfyrplanter fra Harreskov Sande

The

Plan-

Groups I—VIII watered with N-free nutritive solution,

Gruppe I—VIII er vandet med N-fri Næringsopløsning,

The fresh weight before the experiment

| Group <i>Gruppe</i> | Plante No. <i>Plt. Nr.</i> | At the end of the experiment characterised to the naked eye by <i>Ved Forsøgets Afslutning for det blotte Øje karakteriseret ved</i> | Before <i>Før</i> | |
|---|-------------------------------|--|--|------------------------------|
| | | | Fresh weight <i>Friskvægt</i> mg | |
| | | | total <i>ialt</i> | pr. plant <i>pr. Plt.</i> |
| I | 6, 9, 10, 12 | Moderately thick algal layer. No or few dichotomies <i>Middelstærkt Algelaag. Ingen el. kun faa Dichotomier</i> | 2080 | 520 |
| II | 2, 3, 4, 7 | Moderately thick algal layer. Dichotomies fairly abundant... <i>Middelstærkt Algelaag. Ret rigelig Fore- komst af Dichotomier</i> | 2505 | 626 |
| III | 21, 23, 31, 32, 33 | Thick algal layer. Dichotomies sparse <i>Kraftigt Algelaag. Ringe Dichotomi</i> | 985 | 197 |
| IV | 16, 19, 24, 25, 26 | Moderately thick algal layer. Dichotomies fairly abundant... <i>Middelstærkt Algelaag. Forholdsvis rigelig Dichotomi</i> | 1164 | 233 |
| V | 20, 22, 27, 30, 36 | Moderately thick algal layer. No dichotomies <i>Middelstærkt Algelaag. Ingen Dichotomi</i> | 856 | 171 |
| VI | 17, 18, 28, 29, 40 | Very thin algal layer. Dichotomy sparse <i>Meget ringe Algelaag. Ringe Dichotomi</i> | 1240 | 248 |
| VII | 42, 48, 49, 51, 55 | Algal layer above average. No dichotomy <i>Algelaag over Middel. Ingen Dichotomi</i> | 608 | 122 |
| VIII | 50, 52, 53 | Moderately thick algal layer. Dichotomy fairly abundant <i>Middelstærkt Algelaag. Ret rigelig Dichotomi</i> | 488 | 163 |
| Sum total or mean <i>Sum el. Middel:</i> | | | 9926 | 63 |
| IX | 1, 5, 8 | Thick algal layer. Dichotomy sparse <i>Kraftigt Algelaag. Ringe Dichotomi</i> | 2165 | 722 |
| X | 26, 35, 39 | Thick algal layer. Dichotomy very sparse <i>Kraftigt Algelaag. Meget ringe Dichotomi</i> | 701 | 234 |
| XI | 43, 56 | Thick algal layer. Dichotomy moderate <i>Kraftigt Algelaag. Middelstærk Dichotomi</i> | 341 | 170 |
| Sum total or mean <i>Sum el. Middel:</i> | | | 3207 | 141 |

2nd Experiment.

cultivated for two years on N-free sand culture.

dyrket i 2 Aar paa N-fri Sandkultur.

plants.

terne.

groups IX—XI with nitrogenous nutritive solution.

Gruppe IX—XI med N-holdig Næringsopløsning.

is calculated by means of table 4.

| the experiment <i>Forsøget</i> | | | | At the end of the experiment <i>Ved Forsøgets Afslutning</i> | | | | |
|--|-------------------------------|------|------------------------------------|---|------|--|---|---|
| Dry weight <i>Tørvægt</i> mg total <i>ialt</i> | N-content <i>N-Indhold</i> | | Dry weight <i>Tørvægt</i> mg | N-content <i>N-Indhold</i> | | Increase in dry matter <i>Tørstof- tilvækst</i> ‰ | N-content of the sand <i>Sandets N-Indhold</i> ‰ | |
| | mg | ‰ | | mg | ‰ | | ‰ | ‰ |
| 960 | 6.2 | 6.5 | 1260 | 8.5 | 6.7 | 31 | 0.05 | |
| 1155 | 7.5 | 6.5 | 1580 | 3.5 | 2.2 | 37 | 0.04 | |
| 455 | 2.9 | 6.4 | 1020 | 6.1 | 6.0 | 124 | 0.05 | |
| 535 | 3.5 | 6.5 | 1000 | 5.6 | 5.6 | 88 | 0.04 | |
| 395 | 2.5 | 6.3 | 860 | 5.6 | 6.5 | 117 | 0.06 | |
| 575 | 3.7 | 6.4 | 730 | 5.4 | 7.4 | 27 | 0.02 | |
| 280 | 1.8 | 6.4 | 630 | 5.4 | 8.6 | 125 | 0.05 | |
| 225 | 1.5 | 6.7 | 360 | 3.0 | 8.3 | 60 | 0.05 | |
| 4580 | 29.6 | 6.16 | 7440 | 43.1 | 6.41 | 62 | 0.044 | |
| 1005 | 6.5 | 6.5 | 2120 | 11.1 | 5.2 | 110 | 0.06 | |
| 325 | 2.1 | 6.5 | 1190 | 5.9 | 5.0 | 266 | 0.08 | |
| 155 | 1.0 | 6.5 | 800 | 3.2 | 4.0 | 415 | 0.09 | |
| 1485 | 9.6 | 6.5 | 4110 | 20.2 | 4.8 | 177 | 0.077 | |

Table 6.

with self-sown mountain pine plants from Harreskov Sande

Forsøg med selvsaede Bjergfyrplanter fra Harreskov Sande

Nitrogen content of the sand

Sandets Indhold af N

At the beginning of the experiment

Ved Forsøgets Begyndelse

Groups I—VIII and the two control groups were watered with a N-free nutritive solution.

Gruppe I—VIII samt de to Kontrolgrupper er vandet med N-fri Næringsopløsning.

| Group <i>Gruppe</i> | Characterised by <i>Karakteriseret ved</i> | part of the sand <i>Del af Sandet</i> | dry weight <i>Tørvægt</i> g |
|------------------------|---|---|--------------------------------------|
| I | Moderately thick algal layer. No or few dichotomies <i>Middelstærkt Algelag. Ingen el. kun faa Dichotomier</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 290 1340 1630 |
| II | Moderately thick algal layer. Dichotomies fairly abundant <i>Middelstærkt Algelag. Ret rigelig Forekomst af Dichotomier</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 275 1350 1625 |
| III | Thick algal layer. Dichotomy scanty <i>Kraftigt Algelag. Ringe Dichotomi</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 292 1775 2067 |
| IV | Moderately thick algal layer. Dichotomy fairly abundant <i>Middelstærkt Algelag. Forholdsvis rigelig Dichotomi</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 345 1670 2015 |
| V | Moderately thick algal layer. No dichotomy <i>Middelstærkt Algelag. Ingen Dichotomi</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 328 1715 2043 |
| VI | Very thin algal layer. No dichotomy <i>Meglet ringe Algelag. Ringe Dichotomi</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 255 1415 1670 |

2nd Experiment.

cultivated for two years on N-free sand culture.

dyrket i 2 Aar paa N-fri Sandkultur.

at the end of the experiment.

ved Forsøgets Afslutning.

the sand was free from nitrogen.

var Sandet N-frit.

Groups IX—XI were watered with a nitrogenous nutritive solution.

Gruppe IX—XI er vandet med N-holdig Næringsopløsning.

| Selected sample dry weight <i>Udtaget Prøve Tørvægt</i> g | N-content of the sample <i>Prøvens N-Indhold</i> mg | N-content of the sand <i>Sandets N-Indhold</i> | | extra sample <i>Ekstraprøve</i> | | |
|--|--|--|------|--------------------------------------|------------------------|------|
| | | total ialt mg | ‰ | dry weight <i>Tørvægt</i> g | N-content N-Indhold | |
| | | | | | mg | ‰ |
| 20 | 4.6 | 67 | 0.23 | 50 | 9.0 | 0.18 |
| 100 | 1.0 | 13 | 0.01 | 20 | 0.5 | 0.02 |
| | | 80 | 0.05 | | | |
| 20 | 3.3 | 45 | 0.17 | 50 | 10.0 | 0.20 |
| 100 | 1.3 | 18 | 0.01 | | | |
| | | 63 | 0.04 | | | |
| 100 | 28.6 | 84 | 0.29 | | | |
| 50 | 0.6 | 21 | 0.01 | | | |
| | | 105 | 0.05 | | | |
| 50 | 7.4 | 51 | 0.15 | | | |
| 50 | 0.6 | 20 | 0.01 | | | |
| | | 71 | 0.04 | | | |
| 50 | 15.1 | 99 | 0.30 | | | |
| 50 | 0.7 | 24 | 0.01 | | | |
| | | 123 | 0.06 | | | |
| 50 | 1.4 | 7 | 0.03 | | | |
| 50 | 0.8 | 23 | 0.02 | | | |
| | | 30 | 0.02 | | | |

Fortsættes næste Side.

| Group <i>Gruppe</i> | Characterised by <i>Karakteriseret ved</i> | part of the sand <i>Del af Sandet</i> | dry weight <i>Tørvægt</i> g |
|---------------------------------|--|---|--|
| VII | Algal layer above mean. No dichotomy <i>Algelag over Middel. Ingen Dichotomi</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 320 1780 2100 |
| VIII | Moderately thick algal layer. Dichotomy fairly abundant <i>Middelstærkt Algelag. Ret rigelig Dich.</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 240 1020 1260 |
| I—VIII | Sum total or mean <i>Sum eller Middel:</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 2345 12065 14410 |
| IX | Vigorous algal layer. Dichotomy sparse <i>Kraftigt Algelag. Ringe Dichotomi</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 142 680 822 |
| X | Vigorous algal layer. Dichotomy very sparse <i>Kraftigt Algelag. Meget ringe Dichot.</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 226 990 1216 |
| XI | Vigorous algal layer. Dichotomy moderate <i>Kraftigt Algelag. Middelstærkt Dichot.</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 134 670 804 |
| IX—XI | Sum total or mean <i>Sum eller Middel:</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 502 2740 2842 |
| Control <i>Kontrol</i> I | No plants. Moderate algal layer <i>Ingen Planter. Middelstærkt Algelag.</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 198 1080 1278 |
| Control <i>Kontrol</i> II | No plants. Moderate algal layer <i>Ingen Planter. Middelstærkt Algelag.</i> | Upper layer <i>Overlag</i> Lower layer <i>Underlag</i> Total <i>Ialt</i> | 184 1050 1234 |

| Selected sample dry weight <i>Udtaget Prøve Tørvægt g</i> | N-content of the sand <i>Prøvens N-Indhold mg</i> | N-content of the sand <i>Sandets N-Indhold</i> | | extra sample <i>Ekstraprøve</i> | | |
|---|---|--|-------|---------------------------------------|-------------------------------|------|
| | | total ialt mg | ‰ | dry weight <i>Tørvægt g</i> | N-content <i>N-Indhold</i> | |
| | | | | | mg | ‰ |
| 50 | 9.3 | 59 | 0.18 | 50 | 8.5 | 0.17 |
| 50 | 1.1 | 39 | 0.02 | | | |
| | | 98 | 0.05 | | | |
| 50 | 7.6 | 37 | 0.15 | | | |
| 50 | 1.2 | 28 | 0.02 | | | |
| | | 65 | 0.05 | | | |
| | | 449 | 0.191 | | | |
| | | 186 | 0.015 | | | |
| | | 635 | 0.044 | | | |
| 50 | 12.6 | 36 | 0.25 | | | |
| 50 | 0.7 | 15 | 0.01 | | | |
| | | 51 | 0.06 | | | |
| 50 | 17.4 | 79 | 0.35 | | | |
| 50 | 0.8 | 16 | 0.02 | | | |
| | | 95 | 0.08 | | | |
| 50 | 23.9 | 64 | 0.48 | | | |
| 50 | 0.9 | 12 | 0.02 | | | |
| | | 76 | 0.09 | | | |
| | | 179 | 0.357 | | | |
| | | 43 | 0.016 | | | |
| | | 222 | 0.077 | | | |
| 50 | 9.9 | 39 | 0.20 | | | |
| 50 | 1.1 | 24 | 0.02 | | | |
| | | 63 | 0.05 | | | |
| 50 | 7.1 | 26 | 0.14 | | | |
| 50 | 1.0 | 21 | 0.02 | | | |
| | | 47 | 0.04 | | | |

reaction for nitrate), give no manifestation, it may be assumed that small amounts of nitrogen are supplied to the pots in this way if a sufficient quantity of water is required for watering. Thus it will hardly be possible to demonstrate a nitrogen amount of less than 1 mg per litre, though, if 10 litres of the solution have been used, it may have given rise to an addition of perhaps approximately 10 mg of nitrogen. In order to avoid too excessive watering the pots were therefore clad with tin-foil, which ensured that the amount of nitrogen added by the watering would not be of any importance, especially as compared with the amount which may always be supplied from the atmosphere through fixation of NH_3 , or the like. To have a measure of the size of this factor, I inserted in the experiment six control pots without any plants but otherwise equipped entirely as the others and later treated in precisely the same way with watering, etc.

Furthermore, a number of plants were selected from each size class, which were watered with the aforementioned solution but with the addition of 8 g of ammonium nitrate.

All the pots were placed on a cement foundation in the garden of the Agricultural College and with a glass cover built over it.

In the main the plants thrived well after some few of them had died shortly after being planted. They were of a sound green colour. The plants watered with nitrogen developed remarkably long needles, especially in the second year. Thus in one of them (L_5) the needles of the second year were c. 8 cm long against 2.3 cm for the needles the last year before the transplantation.

However, in spite of all our care, in this experiment, also, an infection with algae took place already in the first year, which at the end of the second growth year had formed similar coatings to those mentioned under the previous experiment.

At this time the experiment was interrupted, and the plants taken out. Each individual plant as well as its pot was accurately described.

They were then (with the appertenant pots) divided into groups arranged according to the number of dichotomous mycorrhizae as well as according to the vigour of the algal layer. For each group the dry weight and the nitrogen content of the plants were determined after and before the experiment as

also the nitrogen content of the algal layer of the sand and of the whole quantity of sand. The results of the analyses appear from Tables 5 and 6. In Table 5 the nitrogen content has been computed as 0.30 per cent of the fresh weight (cf. Table 4), and the dry weight for the plants before the experiment is calculated as being equal to $0.462 \times$ the fresh weight as a result of dry weight determinations of the material for Table 4.

That the not inconsiderable increase of the nitrogen content of the sand is not due to the mountain pine plants appears from the conditions of the control pots as compared with groups I—VIII (Table 6). The experiment accordingly confirms A. MÖLLER's result.

Whether dichotomy is abundant or sparse, has had no influence.

Evidently Tables 2, 3, 5 and 6 give no support for the theory that the mycorrhizae of mountain pine in nature should be able to fix the free nitrogen of the air.

As regards the question of a nitrogen-fixing capacity associated with the algal vegetation on the surface of the sand, proofs of such a capacity can hardly be found in Table 6 if it is borne in mind that a nitrogen absorption also took place in the substrate in Melin's aseptically performed nutrition experiments. (Melin 1925, Table 36 and p. 54). It is true that MELIN has not submitted any determination of the nitrogen content of the substrate after the experiments, but as the nitrogen content of a pine or spruce seed is c. 0.3 mg and the plants cultivated without addition of nitrogen at the end of the experiment contained up to 0.84 mg, we can take it for granted that the substrate has absorbed some of the fixed nitrogen of the air.

In addition, Table 6 shows no definite correlation between the intensity of the algal vegetation and the final nitrogen content of the sand.

V. COMPARATIVE INVESTIGATION OF THE SOIL UNDER OLDER MOUNTAIN PINES PLANTED IN RAW HEATH AND THE SOIL OF THE IMMEDIATELY ADJACENT HEATH

In order to elucidate, if possible, on what factors the beneficial effect of the admixed mountain pine on the spruce

cultures in the heath depends, a comparative investigation of the soil under older mountain pines and the soil of the immediately adjoining heath was made in five different Jutlandic plantations.

In addition the soil was investigated under a group of spruces on raw heath which was much more advanced in growth than the surrounding mixed culture of Norway spruce and mountain pine of the same age and planted in the same way on raw heath. (In the group the spruces had perfectly outgrown the pines.) Finally the soil in the poorest part of Harreskov Sande was examined.

The samples were collected by boring in natural deposits with cylindrical tins 10 cm high and containing 265 cm³.

Two samples, one immediately below the other, were taken in each locality, so actually a cylindrical sample to a depth of 20 cm was obtained.

The places in which the samples were taken were selected at a suitable distance from the plant holes, and under mountain pines in such places in which the soil was not covered with heather, grass, or the like, but only by a layer of needles.

At first a hole, 30—35 cm deep, was dug, one side of the hole being cut off perpendicularly by the spade. Near the upper edge of the perpendicular wall the mor, moss, lichens, and loose non-decomposed needles were removed so that the mor was exposed. "Thus it was only the needles which had not yet united with the mor*) layer which were removed".

Then a reversed tin was placed on the layer of mor c. 2 cm from the perpendicular wall, and by turning the tin under pressure a sample was cut out of the soil in its natural position. When the tin was full, it was taken sidewise out across the hole and the lid put on. Subsequently the other tin was turned and filled in exactly the same way.

In the subjoined tables the odd figures indicate the uppermost 10 cm of the soil and the even numbers always the next 10 cm.

Below a summary is given of the localities with appertaining descriptions of the soil, which has reference to the whole depth down to the substratum unaffected by vegetation.

*) Mor is the modern term for raw humus.

Birkebæk Plantation, section II 12.

Raw heath, heather 20—25 cm tall, *Cladina rangiferina*, etc.

Samples 1 and 2.

Description of the soil:

5 cm of heather mor

15 - - leached sand

5 - - black hard-pan.

Then follows stony yellow red-flamed sand.

Birkebæk Plantation, section II 12 (quite close to the preceding locality).

Forty-years old mountain pines planted in raw heath, everywhere closed.

Height c. 5 m.

Samples 3 and 4.

Description of the soil:

6 cm layer of needles

2 - mor

20 - somewhat humus-coloured leached sand.

5 - black hard-pan,

underlain by stony yellow, red-flamed sand,

Birkebæk Plantation, section II 12 (near the two preceding localities).

Forty-years old Norway spruce (a single holm) planted in raw heath.

Height c. 5 m.

Samples 5 and 6.

Description of the soil:

8 cm layer of needles

2 - mor

10 - dark leached sand } interwoven by numerous
spruce roots.

5 - black hard-pan

Then yellow, red-flamed sand penetrated by a few roots.

Birkebæk Plantation, section II 14.

Raw heath with heather 40—50 cm high and *Cladinas*.

Samples 7 and 8.

Description of the soil:

6 cm heather mor

9 - leached sand

3 - black hard-pan, which issues tongues down into the substratum, which consists of light-yellow, slightly stony sand.

Birkebæk Plantation, section II 14 (near the preceding locality).30-years old mountain pine planted in raw heath, height c. 4 m, entirely closed, though with occasional remnants of *Vaccinium vitis idaea*.

Samples 9 and 10.

Description of the soil:

7 cm layer of needles

1 - mor

16 - leached sand

3 - black hard-pan

} with some few roots.

The hard-pan issues tongues down into the substratum, which consists of red-flamed sand.

Birkebæk Plantation, section II 14 (at some distance from the preceding locality).Raw heath, heather 25—30 cm high, *Cladina rangif.*, *Deschampsia flex.*

Samples 11 and 12.

Description of the soil:

10 cm heather mor

27 - leached sand

6 - hard black hard-pan, which issues numerous tongues down into a reddish-yellow stony sand.

Birkebæk Plantation, section 14 (quite close to the preceding locality).Thirty-years old mountain pines planted in raw heath, height c. $3\frac{1}{2}$ m, entirely closed, occasional cowberry shrubs.

Samples 13 and 14.

Description of the soil:

- 6 cm layer of needles
 - 1 - mor
 - 23 - lightly humus-coloured
leached sand with white spots
 - 5—7 - hard, black hard-pan, which issues numerous
tongues down into dark-yellow stone-free sand.
- } scattered roots of
mountain pine.

Harreskov Plantation, section 24.

Raw heath, heather 20—30 cm high, *Cladina rangif.*,
Vaccin. vit. idaea.

Samples 15 and 16.

Description of the soil:

- 10 cm heather mor
- 6 - leached sand
- 1 - dark-brown loose hard-pan.
- Substratum light-yellow stone-free red-flamed sand.

Harreskov Plantation, section 25 (quite close to the preceding locality).

Thirtyfive-years old closed mountain pines, planted
in raw heath, height c. 3 m.

Vegetation: moss and *Vaccin. vit. idaea*.

Samples 17 and 18.

Description of the soil:

- 8 cm layer of needles
 - 2 - mor
 - 26 - leached sand
 - 4 - black hard-pan
- } with a few roots.
- underlain by dark-yellow sand.

Harreskov Plantation, section 15.

Raw heath, heather 30—40 cm high, and *Cladina rangif.*

Samples 19 and 20.

Description of the soil:

- 16 cm black heather mor
- 6 - leached sand
- 1 - black hard-pan
- underlain by dark-brown sand with a fairly large
number of stones.

Harreskov Plantation, section 15 (quite close to the preceding locality).

Twenty-five years old. French mountain pine (*Pinus Mugo rostrata*) height 4—5 m. Here and there heather. Samples 21 and 22.

Description of the soil:

- 3 cm layer of needles
- 1 - mor
- 15 - leached sand
- 3 - loose black hard-pan
- underlain by dark-brown stony sand.

Harreskov Plantation, section 18.

Harreskov Sande. Scattered very low (c. 1 m) and shrubby vegetation of forty-years old mountain pines. Scattered heather and *Cladina*. Sample 22.

Description of the soil:

- No layer of needles
- 1 cm dark mor
- 3 - whitish-reddish leached sand
- underlain by whitish coarse and rather stony sand.

It will be seen that it would have been desirable that the sampling had been carried down to a depth of 30 cm. However, a close inspection of the descriptions of the soil shows that this defect has hardly a one-sided effect on the result as regards the relation between the soil under mountain pines and the soil of the raw heath. In Section II, 13 Birkebæk the hard-pan is not reached in any of the two cases. In Section II, 14 Birkebæk the hard-pan is included in both cases in one locality but in none of the cases in the other locality. In Harreskov Plantation Sect. 15 and 25 the hard-pan was only reached in the raw heath in one of the localities, in the other locality, however, only under mountain pine.

In the laboratory each individual sample was subjected to a careful examination in order to ascertain the nitrogen content, the contents of nitrate and ammonia, the p_H , the content of humus, the pore volume, the water content when the samples were taken in December, the water capacity, the amount of

hygroscopically fixed water, and the absolute dry weight divided by the volume of the sampling cylinder.

The main results are given in Tables 7—17.

The following information may be given about the procedure in the laboratory examination:

The samples taken out by means of tins had a volume of 265 cm³ and were treated in the laboratory immediately after being received in the following way:

First a minor sample was bored out by means of a narrower tin cylinder for determination of the pore volume, water content, and water capacity. The sample was taken centrally in the main sample.

Of the remaining part of the latter, which was mixed energetically, one part was examined for nitrogen, nitrate, nitrite, and ammonia, another part was used for determining the hydrogen ion concentration, and the remainder for determining the humus content and the cubic weight.

In the examination for *nitrate* and *nitrite* CIMMINO's procedure was used (see König 1906), which gives a faint but distinct blue colouring at $\frac{1}{1\,000\,000}$ and a rather strong dark blue colouring at $\frac{1}{100\,000}$. No trace of a blue colouring appeared in any of the samples.

By way of checking, so many drops of a standard solution were added to the soil extract that it contained $\frac{0.5}{1\,000\,000}$ and $\frac{1}{1\,000\,000}$. It proved that with these additions the extracts gave a distinct reaction.

Nor did GRIES's reaction for nitrite (König 1906) give any result.

For *ammonia* the samples were tested with NESSLER's reagent, which by the method indicated by König (1906, p. 809) reacts down to $\frac{1}{1\,000\,000}$. No definite reaction was found in any of the samples.

The determination of *nitrogen* was made according to A. C. ANDERSEN and NORMAN JENSEN'S (1923) modified Kjeldahl method.

The *determination of p_H* was made according to BIILMANN'S quinhydrone method.

The *humus content* was determined as the loss of weight on ignition of absolutely dry material.

Finally, the *pore volume* and the *water capacity* as well as *water contents* and *cubic weights* were determined in the following way:

The aforementioned subsample bored out centrally in the main sample in natural deposition was contained in a tin cylinder 10 cm high with a perforated bottom. The cylinder, the cubic content and weight of which had been determined beforehand, was weighed with its contents. It was then placed in a vertical position with the perforated bottom downwards in a water-basin, which was very slowly filled with water until clear water was seen on the surface of the sample. Then the cylinder was taken out and placed for draining off over a measuring glass. After draining off, it was weighed, and subsequently the cylinder with its contents were dried at room-temperature to a constant weight, which was noted down, and then again at 100° to a constant weight, which was likewise noted down.

From the difference between the absolute dry weight and the water-saturated weight the *water capacity* is obtained; from the difference between the room-dry weight and the absolute dry weight the amount of *hygroscopically fixed water*, and from the difference between the starting weight and the absolute dry weight the *water content when the samples were taken*, the samples having been protected by packing against evaporation during transport.

(Humus substances which might have escaped during the draining over the measuring glass were included in this and in the following cases, their altogether inconsiderable dry weight being ascertained by evaporation).

The absolutely dry subsample was transferred carefully into a measuring glass, and by stirring and a very short treatment with vacuum so much alcohol was added that the surface of the fluid reached the mark indicating the cubic content of the cylinder. The quantity of liquid used divided by this content then yielded the *porosity* expressed in volume percentage.

The *cubic weight* was determined as the ratio between the absolute dry weight in g and the original volume in cm³.

Although the material is actually too small for it, the mean

Table 7. Hydrogen ion concentration.
Brintionkoncentration.

| Sample No. <i>Prøve Nr.</i> | pH | Sample No. <i>Prøve Nr.</i> | pH |
|---|-----|--|-----|
| Raw heath <i>Raa Hede</i> | | Mountain pine <i>Bjergfyr</i> | |
| 1 | 4.6 | 3 | 4.9 |
| 2 | 5.4 | 4 | 4.7 |
| 7 | 5.5 | 9 | 4.0 |
| 8 | 5.0 | 10 | 4.8 |
| 11 | 3.8 | 13 | 4.6 |
| 12 | 4.6 | 14 | 5.1 |
| 15 | 4.1 | 17 | 3.5 |
| 16 | 5.0 | 18 | 5.8 |
| 19 | 4.9 | 21 | 4.6 |
| 20 | 6.0 | 22 | 5.1 |
| Means for uppermost 10 cm (odd numbers) <i>Middeltal for øverste 10 cm (ulige Nr.)</i> | 4.6 | | 4.3 |
| Means for lowermost 10 cm (even numbers) <i>Middeltal for underste 10 cm (tigel Nr.)</i> | 5.2 | | 5.1 |
| Wind-swept sand <i>Afføget Sand</i> | | Good spruce group in raw heath <i>God Grangruppe i raa Hede</i> | |
| 23 | 6.0 | 5 | 4.6 |
| | | 6 | 4.1 |

Means of the differences "mountain pine minus heath" in p_H units
Middeltal af Differenserne „Bjergfyr—Hede“ i p_H Enheder

Uppermost 10 cm — 0.3 ± 0.39
Øverste 10 cm

Lowermost 10 cm — 0.1 ± 0.33
Nederste 10 cm

error has been calculated in most of the tables to serve as a rough indication of what may be concluded from a comparison between the figures obtained from the mountain pine soil and the raw heath soil, respectively.

The calculation was always made by taking for each site compared and each of the two 10 cm high layers of soil the difference between the figure for mountain pine and the figure for raw heath.

The deviation of the individual differences from their means (computed by using the signs) were then regarded as errors, after which the formula

$$m = \pm \sqrt{\frac{v^2}{(n-1) \cdot n}}$$

was used.

Thus m will be equivalent to the mean error we commit if we let the mean of the n individual differences (or the difference between the means of the two series of observations, which is the same thing) express a difference in the relation, under mountain pines and in raw heath respectively.

Under each table the means of the differences: mountain pine minus heath for the uppermost 10 cm and the lowermost 10 cm, respectively, are given. After the means the corresponding values of m are stated. Thus all these figures have only reference to the five sites in which the soil was examined under mountain pines and in an immediately adjacent raw heath, respectively.

It is quite probable that this computation of the mean error gives too unfavourable a picture, for in several of the sites compared the layer of hard-pan was only considered under mountain pine alone or for the heath alone. In mentioning the profiles, we stated that this did not give rise to any one-sidedness in favour of one or the other of the two types of vegetation. But it is obvious that this circumstance may give rise to great fluctuations in the value of the difference "mountain pine minus heath", probably especially as regards the humus and nitrogen contents.

As to the individual tables, the following comments may be made:

Table 7 shows that no definite difference in the *hydrogen ion concentration* under mountain pine and in raw heath, re-

spectively, could be ascertained. The mean of all the samples from the localities compared amounts to 4.45 for the uppermost 10 cm and 5.15 for the lowermost 20 cm respectively. At the same time it will be seen that the one sample from very meagre wind-swept sand shows a p_H of 6.0, while the two samples from the good Norway spruce holm show a mean of 4.35.

The proportion between the p_H values for the uppermost and the lowermost 10 cm would probably seem reasonable, as it is well known that the p_H value increases if we move from the mor down to the substratum (cf. Fr. Weis 1929). It is then natural that the wind-swept sand with its sparse organic life as regards the p_H corresponds more closely to the substratum, while the Norway spruce soil with its large contents of humus (cf. Table 10) corresponds more closely to the mor.

Table 8 provides information about the *nitrogen contents*; the following remarks should, however, be added:

For eight of the samples a double Kjeldahl analysis was made for checking the work. The average difference between two analyses from the same sample was $5\frac{1}{2}$ per cent. and the largest difference 11 per cent. There was no ascertainable systematic difference between the first and the second analysis. When a double analysis was made, the mean of the two results was used.

The high content of nitrogen under the stand with Norway spruce and the low content in Harreskov Sande are remarkable.

On the basis of Table 8 it cannot be definitely decided whether the lower nitrogen content in the uppermost 20 cm of the soil is due to the mountain pines. But there is no doubt that the nitrogen content of the uppermost 10 cm has decreased, and probability suggests that the nitrogen content of the lowermost 10 cm has increased, while that of all the 20 cm has decreased.

In addition to the nitrogen content of the soil, the nitrogen contents of the living plant parts above the soil and of the layers of litter on the surface of the soil should be taken into consideration.

On this problem the following special investigations have been made:

In Section 72 in Nørlund Plantation a sample tree of mountain pine was taken which corresponded as closely as possible to the average tree of the stand. It measured 4.75 m in height, and the diameter was 5.5 cm at a height of 1.3 m.

Table 8. Nitrogen content.

Kvælstofindhold.

| Sample No. Prøve Nr. | N-content per 100 cm ³ N-Indhold pr. 100 cm ³ mg | Total N-content for each site (cylinder 200 cm ³ 20 cm high) Summa N-Indhold for hver Lokalitet (Cylinder 200 cm ³ , 20 cm høj) mg | Sample No. Prøve Nr. | N-content per 100 cm ³ N-Indhold pr. 100 cm ³ mg | Total N-content for each site Summa N-Indhold for hver Lokalitet mg |
|--|--|--|--|--|---|
| Raw heath <i>Raa Hede</i> | | | Mountain pine <i>Bjergfyr</i> | | |
| 1 | 192 | | 3 | 147 | |
| 2 | 29 | <u>221</u> | 4 | 108 | <u>255</u> |
| 7 | 288 | | 9 | 173 | |
| 8 | 111 | <u>399</u> | 10 | 73 | <u>246</u> |
| 11 | 290 | | 13 | 270 | |
| 12 | 53 | <u>343</u> | 14 | 41 | <u>311</u> |
| 15 | 232 | | 17 | 192 | |
| 16 | 42 | <u>274</u> | 18 | 49 | <u>241</u> |
| 19 | 245 | | 21 | 171 | |
| 20 | 64 | <u>309</u> | 22 | 166 | <u>337</u> |
| Means for uppermost 10 cm (odd numbers) <i>Middeltal for øverste 10 cm (ulige Nr.)</i> | 250 | | | 190 | |
| Means for lowermost 10 cm (even numbers) <i>Middeltal for nederste 10 cm (lige Nr.)</i> | 60 | | | 87 | |
| Means for whole locality <i>Middeltal for hele Lokaliteten</i> | | <u>309</u> | | | <u>278</u> |
| Wind-swept sand <i>Afføget Sand</i> | | | Good spruce group in raw heath <i>God Grangruppe i raa Hede</i> | | |
| 23 | 20 | | 5 | 386 | |
| | | | 6 | 216 | <u>602</u> |

Means of the differences "mountain pine minus heath" (mg N).

Middeltal af Differenserne „Bjergfyr — Hede“ (mg N).

Uppermost 10 cm — 60 ± 16

Øverste 10 cm

Lowermost 10 cm + 27 ± 27

Nederste 10 cm

All 20 cm — 31 ± 34

Alle 20 cm

Three slices of the *stem* were cut out with a saw at heights above the ground corresponding to $\frac{1}{6}$, $\frac{3}{6}$ and $\frac{5}{6}$ of the height of the tree, and the nitrogen contents were determined by the Kjeldahl analysis to be 0.16, 0.18 and 0.36 per cent of the dry weight respectively. The diameters of the slices were 62, 48 and 22 mm.

If a nitrogen percentage valid for the entire above-ground body of wood is to be computed, the weight given to the percentages for the individual slices must be proportional to the squares of their diameters; however, since the uppermost slice is not only to represent the stem, but also a number of living and dead branches, the percentage of this slice should have its weight further increased, according to observations in nature by multiplication with 3.

In this way we arrive at a common nitrogen percentage of 0.21, which we will consider as approximately valid also for the other stands in question.

The average volume in the five stands examined may according to yield tables (A. OPPERMAN 1916) be fixed at c. 80 m³ per ha, which corresponds to c. 40 t absolutely dry wood per ha, which again gives c. 80 kg N per ha or 8 mg N per 10 cm² of the surface of the soil.

The dry weight of the *green needles of mountain pine* per square meter of the area covered with pines must be assumed to be about the same as for Scots pine, viz. c. 500 g (TIREN 1927, AMILON 1925).

The nitrogen content of green mountain pine needles was found by BORNEBUSCH (1939) to be c. 0.5 per cent.

However, in samples of green mountain pine needles from Section 380 in Kompedal Plantation I found (1945) a nitrogen content of 1.23 per cent of the dry weight*).

Quite possibly the nitrogen content of the needles may fluctuate with the more or less favourable growth conditions. I consider it most correct, however, to use here my own results

*) In the same section the nitrogen content of needles from a Norway spruce which was still at a standstill and a Norway spruce which had started growth again after the period of arrest, was found to be 1.21 and 1.23 per cent respectively.

The culture was performed in 1923 after trenching. The spruce trees have shown arrested growth for about half a score of years. Now the greater number of them have commenced growth, but some parts have not yet started actual growth again, and here the samples of needles were taken.

as a basis, to which correspond 6 mg per 10 cm² of the surface of the soil.

In two samples from the *needle layer* c. 6 cm thick at the bottom in Section 72, Nørlund Plantation, a nitrogen content of 0.97 per cent and 0.83 per cent respectively was found, to which corresponds on an average 20 mg nitrogen per 10 cm². Since in the five stands of mountain pine compared the average thickness of the layer of needles etc. was precisely 6 cm, their average nitrogen contents above the surface of the ground may be fixed at $20 + 6 + 8 = 34$ mg per 10 cm² of the surface.

In order to find out the *nitrogen contents above the surface of raw heath soil* two samples from a raw heath immediately adjacent to Section 72, containing both heather, moss, and lichens, etc., and the waste of these plants were examined. Contents of 25 and 30 mg nitrogen per 10 cm² of the surface of the ground were found.

Thus it can be established that the material examined gives no indication of an increase of the total amount of nitrogen in an area which has been covered by a growth of mountain pine for about four decades.

Now it may, of course, be maintained that an investigation of the soil to a greater depth than 20 cm might possibly have shown an increase, as it may be supposed that part of the nitrogen liberated from the humus owing to the increased decomposition would have diffused down to greater depths and would still be found there.

Considering that the mountain pine vegetation on these poor soils was greatly in need of all available nutrients, it does not seem probable, however, that any great quantity of the liberated nitrogen should have escaped utilisation. The highly spreading roots of the mountain pine are abundantly represented along the surface of the hard-pan, where they may probably have absorbed all available nitrogen compounds that have diffused down, if they have not already been bound by the also very hungry microflora.

Table 9 shows the *nitrogen contents in per mille of dry weight* and reflects fairly closely the conditions according to Table 8, which means that the cubic weights have not changed materially owing to the different character of the vegetation (cf. also Table 16).

Table 9. Nitrogen content in per mille of dry weight
N-Indhold i pro mille af Tørvægt.

| Sample No. <i>Prøve Nr.</i> | N-content <i>N-Indhold</i> | Sample No. <i>Prøve Nr.</i> | N-content <i>N-Indhold</i> |
|--|-------------------------------|--|-------------------------------|
| Raw heath <i>Raa Hede</i> | | Mountain pine in raw heath <i>Bjergfyr i raa Hede</i> | |
| 1 | 1.7 | 3 | 1.3 |
| 2 | 0.2 | 4 | 0.7 |
| 7 | 3.1 | 9 | 1.5 |
| 8 | 0.7 | 10 | 0.4 |
| 11 | 2.8 | 13 | 2.5 |
| 12 | 0.3 | 14 | 1.4 |
| 15 | 2.1 | 17 | 2.9 |
| 16 | 0.3 | 18 | 0.3 |
| 19 | 2.0 | 21 | 1.4 |
| 20 | 0.4 | 22 | 1.2 |
| Means for uppermost 10 cm (odd numbers) <i>Middeltal for øverste 10 cm (ulige Nr.)</i> | 2.3 | | 1.9 |
| Means for lowermost 10 cm (even numbers) <i>Middeltal for nederste 10 cm (lige Nr.)</i> | 0.4 | | 0.8 |
| Wind-swept sand <i>Afføget Sand</i> | | Good spruce group in raw heath <i>God Grangruppe i raa Hede</i> | |
| 23 | 0.1 | 5 | 9.4 |
| | | 6 | 1.6 |

Means of the differences "mountain pine minus heath" (N i ‰)

Middeltal af Differenserne „Bjergfyr — Hede“ (N i ‰)

Uppermost 10 cm — 0.4 ± 0.38

Øverste 10 cm

Lowermost 10 cm + 0.4 ± 0.81

Nederste 10 cm

Table 10. Humus content.

Humusindhold.

| Sample No. <i>Prøve Nr.</i> | Humus in per cent of dry weight <i>Humus i pCt. af Tørvægt</i> | Absolute humus quan- tity in 100 cm ³ <i>Absolut Humusmængde i 100 cm³</i> g | Sample No. <i>Prøve Nr.</i> | Humus in per cent of dry weight <i>Humus i pCt. af Tørvægt</i> | Absolute humus quan- tity in 100 cm ³ <i>Absolut Humusmængde i 100 cm³</i> g |
|--|---|--|---|---|--|
| Raw heath <i>Raa Hede</i> | | | Mountain pine <i>Bjergfyr</i> | | |
| 1 | 17.7 | 19.5 | 3 | 14.5 | 16.2 |
| 2 | 1.8 | 3.0 | 4 | 5.8 | 9.0 |
| | | <u>22.5</u> | | | <u>25.2</u> |
| 7 | 21.6 | 19.9 | 9 | 11.0 | 13.1 |
| 8 | 5.1 | 7.5 | 10 | 3.9 | 6.4 |
| | | <u>27.4</u> | | | <u>19.5</u> |
| 11 | 18.2 | 19.1 | 13 | 18.2 | 19.3 |
| 12 | 2.8 | 4.7 | 14 | 2.5 | 4.1 |
| | | <u>23.8</u> | | | <u>23.4</u> |
| 15 | 12.2 | 13.5 | 17 | 25.1 | 16.8 |
| 16 | 1.9 | 3.0 | 18 | 1.9 | 2.9 |
| | | <u>16.5</u> | | | <u>19.7</u> |
| 19 | 13.4 | 16.6 | 21 | 6.9 | 8.4 |
| 20 | 3.4 | 5.3 | 22 | 5.9 | 8.2 |
| | | <u>21.9</u> | | | <u>16.6</u> |
| Means for uppermost 10 cm (odd numbers) <i>Middeltal for øverste 10 cm (ulige Nr.)</i> | 13.8 | 17.7 | | 12.6 | 14.7 |
| Means for lowermost 10 cm (even numbers) <i>Middeltal for nederste 10 cm (lige Nr.)</i> | 2.5 | <u>4.7</u> | | 3.3 | <u>6.1</u> |
| Means for all 20 cm <i>Middeltal for alle 20 cm</i> | | 22.4 | | | 20.9 |
| Wind-swept sand <i>Afføget Sand</i> | | | Good spruce group in raw heath <i>God Grangruppe i raa Hede</i> | | |
| 23 | 0.8 | 1.5 | 5 | 55.2 | 22.6 |
| | | | 6 | 12.0 | 15.8 |
| | | | | | <u>38.4</u> |

For the absolute humus quantity in g per 100 cm³ the mean of the differences "mountain pine minus heath" is:

*For absolut Humusmængde i g pr. 100 cm³ er Middeltallet af
Differenserne „Bjergfyr — Hede“:*

Uppermost 10 cm — 3.0 ± 2.1
Øverste 10 cm

Lowermost 10 cm + 1.4 ± 1.3
Nederste 10 cm

All 20 cm — 0.8 ± 1.4

As regards the *humus content* Table 10, on the analogy of the conditions for nitrogen, shows a tendency to a reduction for the uppermost and an increase for the lowermost 10 cm, with a slight decrease for all the 20 cm. This tendency seems natural enough if compared with the descriptions of the profiles, which show a decrease in the thickness of the mor layer and sometimes a darker colour of the leached sand under mountain pines. It is true that no definite conclusion can be drawn from the calculation of the mean error; but more especially in this case this will be unfavourably influenced by the fact that the hard-pan is now included, now omitted, in the 20 cm investigated.

Corresponding entirely to the relations in the case of nitrogen, the humus content of the soil under the stand with Norway spruce is remarkably high, and strikingly low in the wind-swept sand.

Table 11 represents the *relation between the nitrogen and humus contents*. It agrees with the probability that the proportion is a little higher in the uppermost 10 cm in raw heath soil than under mountain pines, where it must be assumed that the nitrogen consumption of the vegetation has been greatest, while conversely in the next 10 cm the proportion is somewhat greater under mountain pines, where some humus particles under decomposition have been washed down; however, as shown by the mean error in the difference between mountain pine and raw heath, the material permits no definite conclusions.

It might have been expected that the ratio $\frac{N}{\text{Humus}}$ would be fairly constant from one locality to the other, but this is not the case.

The ratio shows rather great and for the time being inexplicable fluctuations.

Table 12 gives information about the *pore volume*.

There is a vague tendency towards a greater pore volume in the uppermost 10 cm for mountain pine, while no difference can be observed for the next 10 cm. Under the stand with Norway spruce the pore volume is remarkably great, especially in the uppermost 10 cm, while in Harreskov Sande it is remarkably small.

As regards the *water content* Table 13 shows definitely that when the samples were taken on December 18th, the water

Table 11. Relation between nitrogen and humus.

Forholdet mellem Kvælstof og Humus.

| Sample No. <i>Prøve Nr.</i> | $\frac{N}{\text{humus}}$ | Sample No. <i>Prøve Nr.</i> | $\frac{N}{\text{humus}}$ |
|--|--------------------------|--|--------------------------|
| Raw heath <i>Raa Hede</i> | | Mountain pine in raw heath <i>Bjergfyr i raa Hede</i> | |
| 1 | 0.0098 | 3 | 0.0091 |
| 2 | 97 | 4 | 120 |
| 7 | 145 | 9 | 132 |
| 8 | 148 | 10 | 114 |
| 11 | 152 | 13 | 140 |
| 12 | 113 | 14 | 100 |
| 15 | 172 | 17 | 114 |
| 16 | 140 | 18 | 168 |
| 19 | 147 | 21 | 204 |
| 20 | 121 | 22 | 203 |
| Means for uppermost 10 cm (odd numbers) <i>Middeltal for øverste 10 cm (ulige Nr.)</i> | 143 | | 136 |
| Means for lowermost 10 cm (even numbers) <i>Middeltal for nederste 10 cm (lige Nr.)</i> | 124 | | 141 |
| Wind-swept sand <i>Afføget Sand</i> | | Good spruce group in raw heath <i>God Grangruppe i raa Hede</i> | |
| 23 | 134 | 5 | 170 |
| | | 6 | 137 |

Means of the differences "mountain pine minus heath"

*Middeltal af Differenserne „Bjergfyr — Hede“*Uppermost 10 cm — 7 ± 18
*Øverste 10 cm*Lowermost 10 cm + 17 ± 20
Nederste 10 cm

Table 12. *Determination of pore volume*
by means of alcohol and vacuum exsiccator.

Porevolumenbestemmelse
med Alkohol og Vacuumeksikator.

| Sample No. <i>Prøvedaase Nr.</i> | Pore volume in per cent <i>Porevolumen i Procent</i> | Sample No. <i>Prøvedaase Nr.</i> | Pore volume in per cent <i>Porevolumen i Procent</i> |
|--|--|--|--|
| Raw heath <i>Raa Hede</i> | | Mountain pine <i>Bjergfyr</i> | |
| 1 | 62 | 3 | 64 |
| 2 | 49 | 4 | 49 |
| 7 | 68 | 9 | 61 |
| 8 | 51 | 10 | 46 |
| 11 | 58 | 13 | 64 |
| 12 | 47 | 14 | 47 |
| 15 | 61 | 17 | 81 |
| 16 | 48 | 18 | 49 |
| 19 | 57 | 21 | 62 |
| 20 | 48 | 22 | 55 |
| Means for uppermost 10 cm (odd numbers) <i>Middeltal for øverste 10 cm (ulige Nr.)</i> | 61 | | 66 |
| Means for lowermost 10 cm (even numbers) <i>Middeltal for nederste 10 cm (lige Nr.)</i> | 49 | | 49 |
| Wind-swept sand <i>Afføget Sand</i> | | Good spruce group in raw heath <i>God Grangruppe i raa Hede</i> | |
| 23 | 42 | 5 | 85 |
| | | 6 | 53 |

Means of the differences "mountain pine minus heath"

Middeltal af Differenserne „Bjergfyr — Hede“

Uppermost 10 cm + 5 ± 4.4

Øverste 10 cm

Lowermost 10 cm 0 ± 1.9

Nederste 10 cm

Table 13. *Water content on collection of samples on December 18th expressed in volume percentage.*

Vandindhold ved Udtagelsen ^{18/12} af Jordprøverne udtrykt i Volumenprocent.

| Sample No. <i>Prøve Nr.</i> | Water content <i>Vandindhold</i> | Sample No. <i>Prøve Nr.</i> | Water content <i>Vandindhold</i> |
|--|-------------------------------------|--|-------------------------------------|
| Raw heath <i>Raa Hede</i> | | Mountain pine <i>Bjergfy</i> | |
| 1 | 35.6 | 3 | 34.3 |
| 2 | 10.5 | 4 | 27.8 |
| | <u>46.1</u> | | <u>62.1</u> |
| 7 | 43.2 | 9 | 21.9 |
| 8 | 10.9 | 10 | 13.8 |
| | <u>54.1</u> | | <u>35.7</u> |
| 11 | 44.4 | 13 | 32.9 |
| 12 | 15.1 | 14 | 12.5 |
| | <u>59.5</u> | | <u>45.4</u> |
| 15 | 38.6 | 17 | 26.6 |
| 16 | 13.1 | 18 | 6.7 |
| | <u>51.7</u> | | <u>33.3</u> |
| 19 | 42.0 | 21 | 27.3 |
| 20 | 20.4 | 22 | 21.3 |
| | <u>62.4</u> | | <u>48.6</u> |
| Mean of uppermost 10 cm (odd numbers) <i>Middel af øverste 10 cm (ulige Nr.)</i> | 40.8 | | 28.6 |
| Mean of lowermost 10 cm (even numbers) <i>Middel af nederste 10 cm (lige Nr.)</i> | 14.0 | | 16.4 |
| Mean of all 20 cm <i>Middeltal for alle 20 cm</i> | 27.4 | | 22.5 |
| Wind-swept sand <i>Afføget Sand</i> | | Good spruce group in raw heath <i>God Grangruppe i raa Hede</i> | |
| 23 | 9.4 | 5 | 36.4 |
| | | 6 | 32.2 |
| | | | <u>68.6</u> |

Means of the differences "mountain pine minus heath"

Middeltal af Differenserne „Bjergfy – Hede“

Uppermost 10 cm — 12.2 ± 3.2
Øverste 10 cm

Lowermost 10 cm + 2.4 ± 4.0
Nederste 10 cm

All 20 cm — 4.9 ± 3.5
Alle 20 cm

Table 14. Water capacity expressed in volume percentage.
Vandkapacitet udtrykt i Volumenprocent.

| Sample No. <i>Prøve Nr.</i> | Water- capacity <i>Vandkapacitet</i> | Sample No. <i>Prøve Nr.</i> | Water capacity <i>Vandkapacitet</i> |
|--|--|--|---|
| Raw heath <i>Raa Hede</i> | | Mountain pine <i>Bjergfyr</i> | |
| 1 | 53.3 | 3 | 48.4 |
| 2 | 34.7 | 4 | 37.0 |
| | <u>88.0</u> | | <u>85.4</u> |
| 7 | 64.0 | 9 | 57.8 |
| 8 | 43.5 | 10 | 42.9 |
| | <u>107.5</u> | | <u>100.7</u> |
| 11 | 56.8 | 13 | 58.8 |
| 12 | 31.4 | 14 | 34.4 |
| | <u>88.2</u> | | <u>93.2</u> |
| 15 | 59.6 | 17 | 60.9 |
| 16 | 41.5 | 18 | 26.3 |
| | <u>101.1</u> | | <u>87.2</u> |
| 19 | 49.8 | 21 | 56.0 |
| 20 | 43.8 | 22 | 49.5 |
| | <u>93.6</u> | | <u>105.5</u> |
| Mean of uppermost 10 cm (odd numbers) <i>Middel af øverste 10 cm (ulige Nr.)</i> | 56.7 | | 56.4 |
| Mean of lowermost 10 cm (even numbers) <i>Middel af nederste 10 cm (lige Nr.)</i> | 39.0 | | 38.0 |
| Mean of all 20 cm <i>Middel af alle 20 cm</i> | 47.9 | | 47.2 |
| Wind-swept sand <i>Afføget Sand</i> | | Good spruce group in raw heath <i>God Grangruppe i raa Hede</i> | |
| 23 | 27.6 | 5 | 72.0 |
| | | 6 | 50.8 |
| | | | <u>122.8</u> |

Means of the differences "mountain pine minus heath"

Middeltal af Differenserne „Bjergfyr - Hede“

Uppermost 10 cm — 0.3 ± 2.3
Øverste 10 cm

Lowermost 10 cm — 1.0 ± 3.7
Nederste 10 cm

All 20 cm — 0.7 ± 2.1
Alle 20 cm

Table 15. *Hygroscopically fixed water*
expressed in volume percentage.

Hygroskopisk bundet Vand udtrykt i Volumenprocent.

| Sample No. <i>Prøve Nr.</i> | Hygroscopical water <i>hygroskopisk Vand</i> | Sample No. <i>Prøve Nr.</i> | Hygroscopical water <i>hygroskopisk Vand</i> |
|---|--|--|--|
| Raw heath <i>Raa Hede</i> | | Mountain pine <i>Bjergfyr</i> | |
| 1 | 2.1 | 3 | 1.3 |
| 2 | <u>0.4</u> | 4 | <u>1.3</u> |
| | 2.5 | | 2.6 |
| 7 | 2.6 | 9 | 1.4 |
| 8 | <u>1.2</u> | 10 | <u>0.5</u> |
| | 3.8 | | 1.9 |
| 11 | 2.2 | 13 | 1.7 |
| 12 | <u>0.5</u> | 14 | <u>0.4</u> |
| | 2.7 | | 2.1 |
| 15 | 2.2 | 17 | 1.8 |
| 16 | <u>0.8</u> | 18 | <u>0.8</u> |
| | 3.0 | | 2.6 |
| 19 | 2.6 | 21 | 1.6 |
| 20 | <u>0.9</u> | 22 | <u>1.1</u> |
| | 3.5 | | 2.7 |
| Means of uppermost 10 cm (odd numbers) <i>Middel af øverste 10 cm (ulige Nr.)</i> | 2.3 | | 1.6 |
| Means of lowermost 10 cm (even numbers) <i>Middel af nederste 10 cm (lige Nr.)</i> | 0.8 | | 0.8 |
| Means of all 20 cm <i>Middel af alle 20 cm</i> | 1.6 | | 1.2 |
| Wind-swept sand <i>Afføget Sand</i> | | Good spruce group in raw heath <i>God Grangruppe i raa Hede</i> | |
| 23 | 0.5 | 5 | 2.5 |
| | | 6 | <u>1.6</u> |
| | | | 4.1 |

Means of the differences "mountain pine minus heath"

Middeltal af Differenserne „Bjergfyr — Hede“

Uppermost 10 cm — 0.7 ± 0.5
Øverste 10 cm

Lowermost 10 cm + 0.0 ± 0.8
Nederste 10 cm

All 20 cm — 0.4 ± 0.2
Alle 20 cm

Table 16. *Cubic weight of the samples*

when absolutely dry = $\frac{\text{dry weight g}}{\text{cubic content of sampling cylinder cm}^3}$

$\text{Rumvægt af de udtagne Jordprøver}$
ved absolut Tørhed = $\frac{\text{Tørvægt g}}{\text{Prøvecylinderens Rumfang cm}^3}$

| Sample No. <i>Prøve No.</i> | Cubic weight <i>Rumvægt</i> | Sample No. <i>Prøve No.</i> | Cubic weight <i>Rumvægt</i> |
|--|--------------------------------|--|--------------------------------|
| Raw heath <i>Raa Hede</i> | | Mountain pine <i>Bjergfyr</i> | |
| 1 | 1.10 | 3 | 1.11 |
| 2 | 1.65 | 4 | 1.55 |
| 7 | 0.92 | 9 | 1.19 |
| 8 | 1.48 | 10 | 1.64 |
| 11 | 1.05 | 13 | 1.06 |
| 12 | 1.65 | 14 | 1.63 |
| 15 | 1.12 | 17 | 0.67 |
| 16 | 1.58 | 18 | 1.54 |
| 19 | 1.24 | 21 | 1.22 |
| 20 | 1.55 | 22 | 1.38 |
| Means of uppermost 10 cm (odd numbers) <i>Middeltal af øverste 10 cm (ulige Nr.)</i> | 1.09 | | 1.05 |
| Means of lowermost 10 cm (even numbers) <i>Middeltal af nederste 10 cm (lige Nr.)</i> | 1.58 | | 1.55 |
| Means of all 20 cm <i>Middeltal af alle 20 cm</i> | 1.34 | | 1.30 |
| Wind-swept sand <i>Afføget Sand</i> | | Good spruce group in raw heath <i>God Grangruppe i raa Hede</i> | |
| 23 | 1.83 | 5 | 0.41 |
| | | 6 | 1.31 |

Means of the differences "mountain pine minus heath"

Middeltal af Differenserne „Bjergfyr — Hede“

Uppermost 10 cm — 0.04 ± 0.12
Øverste 10 cm

Lowermost 10 cm — 0.03 ± 0.05
Nederste 10 cm

All 20 cm — 0.04 ± 0.05
Alle 20 cm

content of the uppermost 10 cm was substantially larger in the raw heath than under mountain pine, while no difference could be ascertained for the next 10 cm. The water content under the stand with Norway spruce was remarkably high, while it was remarkably low in the wind-swept sand.

According to Table 14 the *water capacity* for the two 10 cm layers seems to be practically the same under mountain pine and in raw heath soil, whereas it is very great under the Norway spruce stand and very small in the wind-swept sand.

The quantity of *hygroscopically fixed water* in the uppermost 10 cm is markedly smaller under mountain pine than in raw heath soil (Table 15), while it is the same in the layer 10-20 cm from the surface. This condition must be assumed to be influenced by the quantity of humus (cf. Table 10), but is, of course, also influenced by the quantity of inorganic colloids and the size of the particles in general. As might be expected, there is an especially large quantity of fixed water under the stand with Norway spruce and a particularly small quantity in the wind-swept sand.

Finally, Table 16 shows that the *cubic weight of the soil* down to a depth of 20 cm seems to be unaffected by the mountain pine vegetation. The small cubic weight under the Norway spruce holm and the great cubic weight in Harreskov Sande are conspicuous.

Considering all the results of the comparative investigations of the soil under mountain pine and in raw heath, the conclusions that may be drawn with probability are as follows:

The mountain pine vegetation seems to be responsible for a mobilization of nitrogen and humus in the uppermost 10 cm of the soil. Part of the mobilized quantities are found again in the layer 10—20 cm below the surface. As regards the nitrogen, however, the greater part of the mobilized quantity has been used for the growth of the mountain pine and recurs in the living mountain pine vegetation and its layer of litter on the ground. No increase in the quantity of nitrogen per areal unit can be demonstrated.

Furthermore, in the uppermost 10 cm of the soil under mountain pines there is a vague tendency towards a greater pore volume and a smaller cubic weight than in raw heath soil (which corresponds fairly well to the above-mentioned mobili-

zation), while both the water content when the samples were taken on December 18th and the quantity of hygroscopically fixed water seem to be smaller.

On the other hand, the layer 10—20 cm from the surface does not seem affected as regards the pore volume, the water content on the collecting of samples, the quantity of hygroscopical water, and the cubic weight.

Similarly, the p_H as well as the water capacity of the whole layer of soil down to a depth of 20 cm seem to be uninfluenced by the nature of the vegetation.

The differences enumerated above in the relations of the soil in raw heath and under 40 years old mountain pines planted in raw heath soil respectively are nearly all by far less considerable than the differences between the soil under the stand with Norway spruce and the wind-swept sand. In the main both the mountain pine soil and the heath soil are inserted between these two extremes, as will appear from Table 17, in which the means for mountain pine and raw heath soils respectively are given, together with the figures from the holm with Norway spruce and the wind-swept sand.

The investigation thus carried out throws some light on the question as to the factors on which the beneficial effect of mountain pine on Norway spruce in heath cultures depends.

Evidently we are not concerned with a more abundant supply of nitrogen to the soil, but with a capacity in the mountain pine to mobilise the nutrients (including nitrogen) of the heath soil and use them for the growth of trees.

At the same time a slight improvement in the physical condition of the uppermost c. 10 cm of the soil under the mountain pine seems to take place, the pore volume increasing a little. That the water content in December is lower than in the heath, no doubt means that the crowns of the trees to a certain extent protect the soil against rainfall and probably also that the water consumption of the mountain pine is greater than that of the heather during the autumn months. On the other hand, it is not certain that the water content in summer is less under mountain pine than in the open heath, since here the shading influence of the mountain pine must be taken into consideration.

Altogether it is probable that the roots of the Norway

Table 17. *Synoptic table.*

| | Goodsprucegroup <i>God Rødgransgruppe</i> | | Mountain pine <i>Bjergfyr</i> | | Heath <i>Hede</i> | | Wind-swept sand <i>Afføget Sand</i> | |
|---------------------------------------|--|------------------|----------------------------------|------------------|----------------------|------------------|--|------------------|
| | upperm. 10 cm | lowerm. 10 cm | upperm. 10 cm | lowerm. 10 cm | upperm. 10 cm | lowerm. 10 cm | upperm. 10 cm | lowerm. 10 cm |
| pH | 4.6 | 4.1 | 4.3 | 5.1 | 4.6 | 5.2 | 6.0 | |
| N mg per 100 cm ³ | 386 | 216 | 190 | 87 | 250 | 60 | 20 | |
| <i>N mg pr. 100 cm³</i> | | | | | | | | |
| Humus g per 100 cm ³ | 22.6 | 15.8 | 14.7 | 6.1 | 17.7 | 4.7 | 1.5 | |
| <i>Humus g pr. 100 cm³</i> | | | | | | | | |
| Pore volume %/..... | 85 | 53 | 66 | 49 | 61 | 49 | 42 | |
| <i>Porevolumen %/</i> | | | | | | | | |
| Water content on sampling (volume %/) | 36.4 | 32.2 | 28.6 | 16.4 | 40.8 | 14.0 | 9.4 | |
| <i>Vandindh. v. Udt. (Volumen %/)</i> | | | | | | | | |
| Water capacity (volume %/) | 72.0 | 50.8 | 56.4 | 38.0 | 56.7 | 39.0 | 27.6 | |
| <i>Vandkapacitet (Vol. %/)</i> | | | | | | | | |
| Hygroscopical water (vol. %/) | 2.5 | 1.6 | 1.6 | 0.8 | 2.3 | 0.8 | 0.5 | |
| <i>hygroskopisk V. (Vol. %/)</i> | | | | | | | | |
| Cubic weight | 0.41 | 1.31 | 1.05 | 1.55 | 1.09 | 1.58 | 1.83 | |
| <i>Rumvægt</i> | | | | | | | | |

spruce will find somewhat better conditions for growth under the mountain pine than in the heath, since here a liberation of nitrogen is taking place which, at any rate owing to the decomposition of the many dead root parts and fallen needles, may be supposed to be of benefit to the Norway spruce.

When the mountain pine has reached a certain height, it is at least certain that it will benefit the Norway spruce by its shelter against the wind and its partial shading, which makes the water economy of the Norway spruce far more favourable without essentially reducing the possibilities of assimilation, since, as is well known, these do not decrease proportionally with the availability of light (BOYSEN JENSEN 1929, 1932; STÅLFELT 1921, 1924).

Summing up all the effects, we may say that the mountain pine gradually creates a forest situation, and it is in this that its influence should be looked for.

VI. SUMMARY

Anatomy.

My own observations, chiefly on sections of mountain pine and Norway spruce, have always shown a typical ectotrophic mycorrhiza, where only such intracellular hyphae were found as more exceptionally penetrate and in most cases kill the outermost cortical cells.

The descriptions of intracellular hyphae and ectendotrophic mycorrhizae by a number of other authors are dealt with, whereby various disagreements between and uncertainties in the results were ascertained. The widely different digestive processes described by PEKLO (1913) and MELIN (1923) do not seem to have been observed by other workers. The ectotrophic mycorrhiza is evidently entirely predominant in nature, and intracellular infection is most frequently seen in weak plants.

Occurrence.

The majority of authors agree that the mycorrhizae of pine and spruce develop most vigorously on good mor soil of the active type, and most poorly on very meagre mor and on typical mull. My observations agree entirely with this.

Thus, for instance, mountain pine plants from an extremely

poor locality (Harreskov Sande) had few and poorly developed dichotomous mycorrhizae as compared with plants from more favourable localities.

Isolation and synthesis experiments,

carried out with positive results by MELIN (c. 1922) and several later workers, have shown that a considerable number of Basidiomycetes are capable of forming mycorrhizae on the same species of tree.

Isolation experiments made by me showed that a number of different fungi contribute to the formation of the external fungal mantle. The species become the less exacting as regards nitrogen the more poorer the site is. Particularly little exacting were some forms from Harreskov Sande, Jutland.

Synthesis experiments made by me with negative results showed surprisingly good growth of non-mycorrhizal mountain pines, as compared with other species of trees, on almost nitrogen-free substrates. At the same time the mountain pines showed surprisingly good growth in a container which was for years oversaturated with water that was freely visible on the surface of the sand, so that the supply of oxygen to the roots must have been minimal. (Figs. 5 and 6).

Nutrition experiments.

A number of nutrition experiments previously performed are recorded and discussed, thus especially those by MELIN (1925), RAYNER (1934, 1936, 1939), MITCHELL, FINN & ROSENDAHL (1937), HATCH (1937), WHITE (1941) and BJÖRKMAN (1942).

While according to the experiments it must be regarded as certain that neither sterile pine or spruce plants nor such as are provided with synthetically produced mycorrhizae can absorb the free nitrogen of the air (at any rate not to an extent of any importance), most of the other conclusions drawn by the said authors are still doubtful, nor do they agree. I. a. it cannot be considered as proved that the mycorrhizae have a special capacity of assimilating organic N-compounds. (cf. tables 35 and 41 quoted from MELIN 1925 on p. [30] and the natural occurrence of mycorrhizae, see p. [17]). Further there cannot yet be said to be any proof that the mycorrhizae of the trees represent a true mutualistic symbiosis, characterized by

a digestion of intracellular hyphae, as is now often stated in text-books.

It must still be regarded as a possible explanation that the mycorrhiza-producing fungi mainly occur as epiphytes on the roots. This is suggested i. a. by their otherwise saprophytic mode of living and the "lack of criticism" in regard to fungal species by which the apparent symbiosis is marked, and the fact that the mycorrhizae of the conifers are most vigorously developed where the saprophytic activity of fungi is liveliest ("sound" conifer mor).

The saprophytic activity of bacteria and fungi is, no doubt, of great importance for the decomposition of the litter and accordingly for the absorption of nutrition by the roots, and this *may* provide a sufficient explanation of the coincidence of a good growth of trees and a profuse development of mycorrhizae, as also of the biologically conditioned effect of conifer inoculation soil a. o. to nurseries.

In order to find out whether mycorrhizae of mountain pine derived from nature might possibly somehow or other (e. g. in connection with bacteria living on or in them) be capable of fixing the free nitrogen of the air, I made repeated and comprehensive experiments with cultivation on nitrogen-free substrates of mountain pine plants from Harreskov Sande. The result was negative. At the same time the low percentage content in the experimental plants even from the start was remarkable (tables 1—6).

In simultaneous experiments with plants produced without mycorrhizae it turned out that the mycorrhizal fungus, at any rate as regards the material employed, did not accompany the seed.

Comparative investigations of the soil under old mountain pines planted in raw heath and the soil of the adjacent heath.

In five selected localities a thorough comparative investigation was made of the uppermost 20 cm of soil under stands of old mountain pines and in the immediately adjoining heath, for the purpose of ascertaining the alterations that might have been caused by the presence of mountain pines for c. 40 years.

A corresponding investigation was also made of the soil under a clump of spruce trees which had grown far in advance of the remaining culture of mixed Norway spruce and mountain

pine of the same age in raw heath soil, — and of the soil in the poorest part of Harreskov Sande (tables 7—17).

The mountain pine vegetation seems to have given rise to a mobilization of nitrogen and humus in the uppermost 10 cm of the soil. Part of the mobilized quantities are probably found again in the layer 10—20 cm below the surface. As regards the nitrogen, however, the greater part of the mobilized quantity seems to have been used for the growth of the mountain pine and recurs in the living mountain pine vegetation and its waste layer on the ground. No increase of the nitrogen quantity per areal unit could be ascertained. (The quantity of the other nutrients was not investigated).

In addition the pore volume in the uppermost 10 cm of the soil under the mountain pines seemed to be a little larger and the cubic weight a little less than in the raw heath, while the water content on collection of the samples on December 18th as well as the quantity of hygroscopically fixed water were decidedly less.

The layer 10—20 cm from the surface, however, did not seem to be affected in regard to the pore volume, the water content at the time of sampling, the quantity of hygroscopic water, and the cubic weight.

For the whole layer of soil down to a depth of 20 cm both the p_H and the water capacity seemed likewise to be unaffected by the character of the vegetation.

The differences in the relations of the soil thus enumerated, in raw heath soil and under mountain pines planted in raw heath soil respectively, are nearly all of them smaller than the difference between the soil under the spruce stand and in wind-swept sand. In the main both the mountain pine soil and the heath soil range between these two extremes (cf. the survey in table 17).

Evidently the well-known beneficial effect of mountain pine on Norway spruce in heath cultures does not, as assumed by P. E. MÜLLER, depend on an additional supply of nitrogen to the soil, but on a capacity of the mountain pine to make frugal use of the nutrients of the heath soil, including nitrogen, and to utilize them in the growth of trees.

It is probable that the roots of the Norway spruce will find somewhat better growth conditions under mountain pines

than in the heath soil, because here a liberation of nitrogen takes place which at any rate through the decomposition of the many dead root parts and fallen needles must be assumed to be of benefit to the Norway spruce.

When the mountain pine has attained a certain height, it is a fact that it will be beneficial to the Norway spruce owing to its shelter against the wind and its side shade, which make the water economy of the Norway spruce more favourable without correspondingly reducing its possibilities of assimilation. A forest environment has been created.

Concluding remarks.

As will appear from the survey of the investigations hitherto made, there is no certain indication that the special capacity of the mountain pine (or the Scots pine) to grow under difficult conditions should depend on its mycorrhizae. The microscopic picture of the mycorrhizae of the pine and the spruce is essentially the same, and it is largely the same species of fungi which produce the mycorrhizae.

Even if we assume that mycorrhizae in general have a special power to utilize organic nitrogen compounds, it is difficult to understand why the fungi should be more active in this respect in the pine than in the spruce. We know, however, that the water consumption of the pine is very small and its requirement of mineral nutrition essentially smaller than that of the spruce (BURGER 1941, WOLFF 1874, 1880), and in my experiments the mountain pine has been especially little exacting as regards nitrogen nutrition as well as oxygen supply to the roots.

It must therefore be most natural to explain the special pioneering capacity of the mountain pine in heath cultures solely by its modest requirements, which make its start possible even under the most unfavourable conditions and subsequently, when the ground is covered, give rise to an improved decomposition of the humus substances.

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RESUMÉ.

MYCORRHIZER OG KVÆLSTOFASSIMILATION,
MED SÆRLIGT HENBLIK PAA
BJERGFYR OG RØDGRAN.*Indledning.*

Forarbejderne til nærværende Arbejde paabegyndtes i 1920, da Hedeselskabet tilskyndet af P. E. MÜLLER udskrev en Prisopgave med det Formaal at faa det Spørgsmaal besvaret, om Bjergfyrrer og Skovfyrrer ved Hjælp af deres dichotome*) Mykorrhizer er i Stand til at optage Luftens frie Kvælstof, hvorved evt. Bjergfyrrerens gavnlige Virkning paa Rødgran i Hedekulturerne kunde forklares.

Forfatteren indleverede i to Tempi en foreløbig Besvarelse, som belønnedes med $\frac{2}{3}$ af Prisen og er omtalt i Hedeselskabets Tidsskrift 1923 p. 159, hvor det i Hovedsagen kun nævnes, at det ikke er lykkedes ved Forsøg at paavise nogen Kvælstofbinding. Besvarelsen fremhævede iøvrigt paa Basis af Undersøgelser, at da der fra Svampeskeden fortrinsvis har kunnet isoleres meget kvælstofnøjsomme Svampearter, og da saavel sterilt frembragte Bjergfyrplanter som Bjergfyrplanter hentede i Naturen klarer sig med overordentlig smaa Kvælstofmængder, maa Kvælstofbindingsteorien alene derfor anses for usandsynlig.

Arbejdet har siden med Afbrydelser været fortsat i udvidet Form, og det samlede Resultat forelægges her med Hedeselskabets velvillige Tilladelse.

Anatomiske Forhold.

Mine egne iagttagelser, hovedsagelig paa Snit af Bjergfyr og Rødgran, har altid vist en typisk ektotrof Mykorrhiza, hvor der af intracellulære Hyfer kun fandtes saadanne, der mere undtagelsesvis gennemløber og oftest dræber de yderste Barkceller. (Fig. 1—4)

En Række andre Forfatteres Beskrivelser af intracellulære Hyfer og ektendotrofe Mykorrhizer er gennemgaaet, hvorved

*) P. E. MÜLLER (1903) skelner hos Fyrrer mellem gaffelformet (dichotome) og klaseformigt forgrenede (racemose) Mykorrhizer.

der er konstateret forskellige indbyrdes Uoverensstemmelser og Usikkerheder i Resultaterne. De af PEKLO (1913) og MELIN (1923) skildrede meget forskellige Fordøjelsesprocesser synes ikke iagttaget af andre. Den ektotrofe Mykorrhiza er aabenbart den i Naturen ganske dominerende, og intracellulær Infektion ses oftest paa svage Planter.

Forekomst.

Flertallet af Forfattere er enige om, at Fyrrens og Granens Mykorrhizer udvikler sig kraftigst paa god Morbund af den aktive Type, derimod daarligst paa mager Mor og paa typisk Muldbund. Mine iagttagelser stemmer overens hermed.

Eksempelvis havde Bjergfyrplanter fra yderst mager Lokaltet (Harreskov Sande) faa og daarligt udviklede dichotome Mycorrhizer sammenlignet med Planter fra bedre Lokaltet (S. 17).

Isolerings- og Syntheseforsøg

udført med positivt Resultat af MELIN (ca. 1922) med flere senere Forskere har vist, at et betydeligt Antal Basidiomycet-Arter er i Stand til at danne Mykorrhizer paa samme Træart.

Af mig udførte Isoleringsforsøg viste, at en Mængde forskellige Svampe medvirker i Dannelsen af den udvendige Svampeskede. Arterne bliver desto nøjsommere med Hensyn til Kvælstof, jo magrere Lokalteten er. Ganske særlig nøjsomme var nogle Former fra Harreskov Sande, Jylland.

Af mig med negativt Udfald anstillede Syntheseforsøg viste overraskende god Vækst af mycorrhizafri Bjergfyr, sammenlignet med andre Træarter, paa næsten N-frit Substrat. Samtidig viste Bjergfyrren forbavsende god Vækst i en enkelt Beholder, der gennem flere Aar var overmættet med Vand, som stod frit frem paa Sandets Overflade, saaledes at Tilgangen af Ilt til Rødderne maa have været minimal. (Fig. 5 og 6).

Ernæringsforsøg.

En Række tidligere Ernæringsforsøg er refereret og drøftet, herunder navnlig MELINS (1925), RAYNERS (1934, 1936, 1939), MITCHELL, FINN & ROSENDAHLS (1937), HATCH' (1937), WHITES (1941) og BJÖRKMANS (1942).

Medens det efter Forsøgene maa betragtes som sikkert, at hverken sterile eller med syntetisk frembragte Mykorrhizer

forsynede Fyrre- eller Granplanter kan optage Luftens frie N (i alt Fald ikke i et Omfang, der har Betydning), er de fleste andre af de omtalte Forfattere dragne Slutninger endnu usikre og heller ikke overensstemmende. Bl. a. kan det ikke betragtes som bevist, at Mykorrhizer har en særlig Evne til at optage organiske N-Forbindelser. (jvf. Tabellerne 31 og 41 citeret fra MELIN 1925 S. [30] og Mykorrhizernes naturlige Forekomst, se S. [17]). Der kan ikke siges at foreligge noget Bevis for, at Træernes Mykorrhizer repræsenterer en ægte mutualistisk Symbiose, karakteriseret af en Fordøjelse af intracellulære Hyfer, saaledes som det nu almindeligt anføres i Lærebøger.

Det maa endnu betragtes som en mulig Forklaring, at de mykorrhizadannende Svampe fortrinsvis optræder som Epifyter paa Rødderne. Herpaa tyder bl. a. deres iøvrigt saprofytiske Levemaade og den „Kritikløshed“ med Hensyn til Svampearter, der præger den tilsyneladende Symbiose, samt det Forhold, at Naaletræernes Mykorrhizer findes bedst udviklet, hvor Svampeflooraens saprofytiske Virksomhed er livligst („sund“ Naaletræ-Mor).

Den saprofytiske Virksomhed af Svampe og Bakterier er utvivlsomt af stor Betydning for Affaldets Omsætning og dermed for Røddernes Næringsoptagelse, og heri kan ligge en tilstrækkelig Forklaring paa Sammenfaldet mellem god Trævækst og rigelig Mykorrhizaudvikling, ligesom paa den biologisk betingede Nytevirkning af Naaletræ-Podejord o. l. til Planteskoler.

For at undersøge, om muligvis i Naturen hentede Mykorrhizer af Bjergfyr paa en eller anden Maade (f. Eks. i Forbindelse med paa eller i dem levende Bakterier) skulde være i Stand til at binde Luftens frie N, anstillede jeg gentagne og omfattende Forsøg med Dyrkning paa N-frit Substrat af Bjergfyrplanter hentede i Harreskov Sande. Resultatet var negativt. Samtidig var det paafaldende, hvor lavt det procentiske Indhold i Forsøgsplanterne var allerede ved Starten (Tab. 1—6).

Under samtlige Forsøg med mykorrhizaløst frembragte Sammenligningsplanter viste det sig, at Mykorrhizasvampen, i alt Fald for det anvendte Materiale, ikke fulgte med Frøet.

Sammenlignende Undersøgelse af Jordbunden under ældre Bjergfyr plantet i raa Hede og Jordbunden paa den tilgrænsende Hede.

Paa 5 udvalgte Lokalteter er foretaget en indgaaende sammenlignende Undersøgelse af de øverste 20 cm tykke Jordlag under ældre Bjergfyr og paa den umiddelbart tilgrænsede Hede

med det Formaal at konstatere de Forandringer, som den ca. 40-aarige Tilstedeværelse af Bjergfyr maatte have bevirket.

En tilsvarende Undersøgelse er tillige foretaget af Jordbunden under en Grangruppe, der var groet langt forud for den øvrige, jævaldrende Kultur af Rødgran og Bjergfyr i raa Hede, samt af Jordbunden i den magreste Del af Harreskov Sande (Tab. 7—17).

Bjergfyrvegetationen synes at have bevirket en Mobilisering af N og Humus i de øverste 10 cm af Jordbunden. En Del af de mobiliserede Mængder genfindes vistnok i Laget 10—20 cm under Overfladen. For Kvælstoffets Vedkommende er dog aabenbart den største Del af det mobiliserede forbrugt til Bjergfyrrens Vækst og genfindes i den levende Bjergfyrvegetation samt dens Affaldslag paa Jorden. Nogen Forøgelse af N-Mængden pr. Arealenhed kunde ikke paavises. (Mængden af de øvrige Næringsstoffer er ikke undersøgt).

Endvidere syntes under Bjergfyr i de øverste 10 cm af Jorden Porevolumen lidt større og Rumvægten lidt mindre end i den raa Hede, medens saavel Vandindholdet ved Prøve-Udtagelsen 18. December som Mængden af hygroskopisk bundet Vand afgjort var mindre.

Derimod syntes Laget 10—20 cm fra Overfladen ret upaa-virket med Hensyn til Porevolumen, Vandindhold ved Udtagelsen, Mængden af hygroskopisk Vand og Rumvægten.

Ligeledes syntes for hele Jordlaget ned til 20 cm Dybde saavel p_H som Vandkapacitet upaa-virket af Vegetationens Art.

De saaledes opregnede Forskelle paa Jordbundens Forhold henholdsvis i raa Hede og under Bjergfyr plantet i raa Hede er saa godt som alle af mindre Størrelse end Forskellene mellem Jordbunden under Granholmen og i afføget Sand. I alt væsentligt ligger baade Bjergfyrjorden og Hedejorden indskudt mellem disse to Yderpunkter. (Jvf. Oversigten Tab. 17).

Øjensynlig er Bjergfyrrens bekendte gavnlige Virkning paa Rødgran i Hedekulturene ikke, som af P. E. MÜLLER formodet, betinget af en Berigelse af Jorden med Kvælstof, men af en Evne hos Bjergfyrren til at tilgodegøre sig Hedejordens Næringsstoffer, herunder Kvælstof, og benytte dem til Trævækst.

Det er sandsynligt, at Rødgranens Rødder vil finde noget bedre Vækstbetingelser under Bjergfyrren end i Hedejorden, fordi der her sker en Kvælstoffrigørelse, som i alt Fald gennem

Dekompositionen af de mange døde Roddele og nedfaldne Naale maa tænkes at komme Rødgranen tilgode.

Naar Bjergfyrren har naaet en vis Højde, er det givet, at den vil gavne Rødgranen ved sit Læ og sin Skygge, som stiller Rødgranens Vandøkonomi gunstigere, uden at det behøver at gaa ud over dens Assimilationsmuligheder. Der er skabt en Skovsituation.

Slutbemærkning.

Der foreligger, som det vil fremgaa af Oversigten over hidtidige Undersøgelser ingen Holdepunkter for, at Bjergfyrrens (eller Skovfyrrens) særlige Evne til Vækst under vanskelige Forhold skulde være betinget af dens Mycorrhizer. Det mikroskopiske Billede af Fyrren og Granens Mycorrhizer er i alt væsentligt det samme, og det er i vid Udstrækning de samme Svampearter, der danner Mycorrhizerne.

Selv om man antager, at Mycorrhizer i Almindelighed har en særlig Evne til Udnyttelse af organiske Kvælstofforbindelser, er det vanskeligt at se, hvorfor Svampene i denne Henseende skulde være mere aktive hos Fyrren end hos Granen. Derimod ved vi, at Fyrrens Vandforbrug og Behov for mineralsk Næring er væsentlig mindre end Granens (BURGER 1941, WOLFF 1874, 1880), og i mine Forsøg har Bjergfyrren vist en ganske særlig Nøjsomhed med Hensyn til saavel Kvælstofnæring som Ilttilgang til Rødderne.

Det maa derfor ligge nærmest at forklare Bjergfyrrens særlige banebrydende Evne i Hedekulturer alene ved dens Nøjsomhed, der muliggør dens Start under selv de vanskeligste Forhold og senere, naar Bunden er dækket, bevirker en forbedret Omsætning af Humusstofferne.
